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Biochemical composition, heavy metal content and their geographic variations of the form species *Nostoc commune* across China

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Abstract

Nostoc commune is a form species. It has been used as a food and herbal medicine for a long time. This study investigated the biochemical composition, heavy metal content and their geographic variations of the *N. commune* colonies, which were sampled from all the main production areas throughout the mainland China. *N. commune* from different areas in China had significant variations in biochemical composition and heavy metal contents. The dry mass of *N.commune* is mainly composed of carbonate rather than protein. Compared to *Nostoc sphaeroides* and wheat, *N. commune* contains low protein,but has higher dietary fibre content. Meanwhile, it has a higher content of Ca and Fe minerals.There were significant features in the biochemical composition and heavy metal content with latitude in China. With the increasing latitude, ash content and lead generally tended to increase, while carbohydrate content tended to decrease. All the samples are polluted by the heavy metals lead, chromium and arsenic, and now all of them are not safe enough for food unless these heavy metals are removed. If the heavy metals Pb, Cr and As are removed, *N.commune* can be good resources of carbonate and protein to human being and animals.

Keywords: nutrients; food safety; geographic variations; latitude; *Nostoc commune*.

Practical Application: *N.commune* can be good resources of carbonate and protein to human being and animals, if the heavy metals would be removed.

1 Introduction

Nostoc is a genus of filamentous cyanobacteria, and it can form macroscopic colonies in terrestrial habitats. *Nostoc commune* is a form species. Together with other two form species, *Nostoc flagelliforme* and *Nostoc sphaeroids*, it is usually considered belonging to a same genetic species (Wright et al., 2001). *N. commune* is used as a food for a long history in many countries, such as China, Japan, Philippine and Mexico (Briones-Nagata et al., 2007). Recently, it is used as an herbal medicine, treating a variety of medical conditions, including inflammation, night blindness, burns, anxiety, and chronic fatigue (Rasmussen et al., 2008). *N. commune* is distributed world widely, from polar valleys to tropic regions, especially those associated with nutrient-poor soils and limestones (Dodds et al., 1995).

The biochemical composition of *N. commune* has long been investigated in the early 1990s (Hori et al., 1990). However, these local and small samples came from small areas might not represent characteristics of the population due to the variations of the samples. It is supposed that the biochemical composition of samples from different regions or different sampling times vary significantly because they are easily influenced by external (environmental conditions) and intrinsic (life history) factors (Briones-Nagata et al., 2007). Differences have been found between samples from different seasons (Hori et al., 1990). However, few studies were conducted to verify their geographic variations, especially in large space scales.

The dry mass of the *N. commune* colony is mainly composed of extracellular polymeric substances (EPS) (Brüll et al., 2000), which usually take negative charges. EPS has been considered very promising as chelating agents for the absorption and adsorption of positively charged heavy metals (De Philippis et al., 2001). With the increasing environmental pollution, it is supposed that the wide resources of *N. commune* are facing up with the risk of heavy metal pollution. It is questionable whether it is still safe enough as human food or herbal medicine nowadays, especially those wild resources grown in heavy metal polluted areas.

China spans large gradients of climate and vegetation, and it shows north-south and east-west gradients in climate, geomorphology and soil substrate materials (Han et al., 2011). It thus provides a good representation of environmental heterogeneity for the study of geographic variation of *N.commune*. Besides, China is facing serious environmental problems in recent years. Various kinds of pollution sources have increased as China has industrialized, which has caused widespread environmental and food safety problems (Zhang et al., 2015). Awareness about the health benefits of foods and nutrition cause to increase the demand for new and healthy food products (Yildiz et al., 2021). In this study, *N.commune* colonies distributed in different regions throughout China were collected and their biochemical composition and heavy metals were measured in order to explore their nutritional characteristics, food safety status and geographic variations throughout China. This study intends to provide geographical basis for wild resource usage of *N. commune* in China.

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2 Materials and methods

2.1 Sample collection and pretreatment

N. commune samples were collected from 30 different counties, distributed in 16 provinces or autonomous regions in China (Figure S1). To minimize the influence of seasonal aspect on the variation of sample characteristics, all the samples were collected in the late spring in 2011 and 2012. In each sampling area, 5 to 10 samples from different microclimates and soil textures were collected to make one composite sample. For each composite sample, more than one kilogram of dry samples was sampled to minimize the influence of different life histories of the samples. All fresh samples were washed with distilled water, air dried, and stored in the desiccators for further analyses.

2.2 Biochemical composition analysis

Crude protein, crude lipid and ash were measured according to the method of Chinese Standard Agency for food as described by Hao et al. (2011) as follows: crude protein was based on the results of crude nitrogen by the method after Kjeldahl; crude lipid was estimated by Soxhlet extraction; Ash content was expressed as the percentage of residue remaining after dry oxidation at 550 ± 25 °C. Carbohydrate was calculated by difference between total dry weight and crude protein, crude lipid and ash. Amino acid compositions of *N. commune* were determined by amino acid analyzer (Hitachi-L8800) according to the methods of Hao et al. (2011). Copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cobalt (Co), selenium (Se), calcium (Ca) were measured following the procedure previously described by Tamasi et al. (2013) with a flame atomic absorption spectrophotometers Perkin-Elmer 5000 and Perkin-Elmer 300 (Perkin-Elmer, Monza, Italy).

2.3 Heavy metal content analysis

Heavy metals including lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), and arsenic (As) were measured in this study. Pb, Cd and Cr were also measured by the flame atomic absorption spectrophotometers (Tamasi et al., 2013). Hg concentrations were analyzed following U.S. EPA Method 1630, including pre-digestion with 0.5% (v/v) 0.2 N bromine monochloride, reduction with hydroxylamine hydrochloride, further reduction with tin chloride, purging of Hg onto gold traps, and quantification of Hg by cold vapor atomic fluorescence spectrometry (CVAFS, Tekran Model 2500 Hg Analyzer, Knoxville, USA) (Rothenberg et al., 2012). For quantification of arsenic content, Dry matter samples were predigested with a mixture of nitric acid and perchloric acid. Arsenic content in the resulting solution was determined by atomic absorption spectrophotometry (model Spectr AA220, Varian Medical Systems, Inc., USA) (Guangwen & Suter, 2011).

2.4 Statistical analyses

Coefficient of variation was used to describe the variation of different samples in biochemical compositions and heavy metal contents. In order to show the decreasing order of different concentrations of minerals and heavy metals, their average concentrations were converted as the base-10 logarith. Pearson Correlation Coefficient was calculated to describe the correlation between biochemical compositions and heavy metals with geographic variables and between biochemical compositions and heavy metals themselves. All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL).

3 Results and discussion

3.1 Biochemical compositions of N.commune in China

N.commune composition vary according to the culturing position. Table 1 shows the results of 32 samples in terms of proteins, lipid, ash, carbohydrate and heavy metals. As shown in Table 1, carbohydrate contributed to the most of the dry weight of *N.commune* and it could catch up to 70% of the dry sample, followed by ash and protein. Lipid content was comparatively very low in *N. commune*. Except for lipid, ash varied much more than carbohydrate, protein and the ratio of carbohydrate to protein. Its coefficient of variation was 29.82%, which was more than two times of carbohydrate (12.55%) and protein (11.74%). Ash content was inversely proportional to protein content ($r = -0.49$, $p = 0.01$) and carbohydrate content ($r = -0.98$, $p = 0.00$). No significant correlation was found between protein content and carbohydrate content ($r = 0.29$, $p = 0.12$).

N. commune had very high concentrations of Ca and Fe, up to 8.53 g kg^{-1} and 1.85 g kg^{-1} dry weight respectively, followed by Mn, Zn, Cu, Co and Se (Table 1). Ca content exceeded potassium content (average 3.93 g kg^{-1}) while Fe content exceeded phosphorus content (0.78 g kg^{-1}) in *N. commune*.

N. commune mainly had 15 kinds of amino acids. The first two more amino acids were aspartic acid (Asp), glutamic acid (Glu), and both of them are delicious amino acids. Neither methionine (Met) nor cystine (Cys) was detected in all the samples (Table 2). Among the amino acids detected, proline (Pro) had the highest coefficient of variation (Table 3).

Compared with *Spirulina* (average 63% protein), *N. commune* had less protein (average 16% protein), so it is not a preferred protein source as human food. On the contrary, it has much higher content of carbohydrate, average 57% in *N. commune* versus 26% in *Spirulina*(Figure 1), which has potential nutrient and medicine value for human body (Guangwen & Suter, 2011). Meanwhile, it has plenty of calcium and iron minerals, and that make them to be one of the richest calcium and iron food. For cyanobacteria, calcium is required for heterocyst differentiation, nitrogen fixation, PS-II activity, and for phosphate uptake (Pandey et al., 1996). It has also been found to exert a protective role against heavy metal toxicity. For example, it was showed that Ca was able to protect the cyanobacterial cells against potassium (K), Maganesium (Mg) and Fe loss caused by heavy metals (Fernandez-Piñas et al., 1997). Our results also showed that there might exist synergy absorption between Ca with other minerals like Co, Zn, Mn, and Se. Dietary intake is the body's main source of Ca, whose absorption is affected by the nature of the chemical matrix of the food source and individual metabolism (Ji et al., 2022), *N.commune* could be one source of Ca for human diet. Fe is one of the most important ingredients of chlorophyll and it acts as a cofactor of many enzymes and even its deficiency leads to the loss of biochemichal pathway in cyanobacteria (Rajeshwari & Rajashekhar, 2011). With greater

Table 1. Descriptive statistics of biochemical compositions, heavy metals contents of *N. commune* sampled from different areas in China.

	Mean value	Maximum value	Minimum value	Standard deviation	Coefficient of variation (%)
Protein (%) (n = 32)	16.35	20.91	11.23	1.92	11.74
Lipid (%) (n = 32)	0.17	0.34	0.02	0.10	58.82
Ash (%) (n = 30)	26.46	49.29	14.9	7.89	29.82
Carbohydrate (%) (n = 30)	57.19	69.85	37.78	7.18	12.55
Carbohydrate/Protein (n = 30)	3.57	5.46	2.72	0.55	15.41
Cu (mg kg^{-1}) (n = 33)	9.12	13.93	5.6	2.15	23.57
Zn (mg kg^{-1}) (n = 33)	34.71	70.39	19.5	12.01	34.60
Fe (mg kg^{-1}) (n = 33)	1,851.32	3,943.63	582.88	1,000.62	54.04
Mn (mg kg^{-1}) (n = 33)	131.51	201.79	74.55	35.97	27.35
Co (mg kg^{-1}) (n = 33)	2.94	5.69	0.46	1.27	43.20
Se (mg kg^{-1}) (n = 33)	0.2	0.27	0.06	0.05	0.25
Ca (mg kg^{-1}) (n = 33)	8,525.01	12,600.00	3,390.6	2,755.4	32.32
Pb (mg kg^{-1}) (n = 33)	15.66	37.59	8.76	6.19	39.53
Cd (mg kg^{-1}) (n = 33)	0.40	0.60	0.25	0.08	20.00
Cr (mg kg^{-1}) (n = 33)	14.74	33.69	6.7	5.78	39.21
Hg (mg kg^{-1}) (n = 33)	0.022	0.081	0.015	0.012	54.54
As (mg kg^{-1}) (n = 33)	4.01	8.05	1.63	1.47	36.66

Table 2. Descriptive statistics of amino acid compositions of *N. commune* (n = 32) sampled from different areas in China.

	Mean value	Maximum value	Minimum value	Standard deviation	Coefficient of variation (%)
Essential amino acids (g/100 g protein)					
Threonine	6.91	7.70	5.44	0.47	6.81
Valine	6.03	7.31	5.27	0.37	6.08
Isoleucine	5.17	5.80	4.64	0.27	5.26
Leucine	7.52	8.33	6.53	0.43	5.73
Phenylalanine	4.90	5.36	4.35	0.23	4.59
Tyrosine	2.09	2.52	1.74	0.20	9.63
Histidine	3.06	4.30	1.74	0.50	16.32
Lysine	3.26	3.73	2.89	0.20	6.18
Arginine	6.73	7.61	5.15	0.53	7.92
Non-essential amino acids (g/100 g protein)					
Aspartic acid	12.61	13.79	9.85	0.83	6.60
Serine	4.64	5.28	3.78	0.30	6.51
Glutamic acid	10.40	11.38	9.22	0.55	5.25
Glycine	5.92	6.55	4.87	0.32	5.36
Alanine	6.88	7.64	5.78	0.40	5.83
Proline	8.81	16.11	4.44	2.25	50.68

Table 3. Correlation coefficients amino acid with geographic variables of *N. commune*.

Correlation coefficients	Latitude	Longitude	Altitude	Distance to the Equator	Distance to this early meridian
IIE	-0.111/0.539	0.148/0.410	-0.374*/0.032	-0.111/0.539	0.168/0.349
LEU	-0.074/0.683	0.122/0.497	-0.355*/0.043	-0..074/0.683	0.121/0.504
LYS	-0.001/0.997	0.087/0.632	-0.277/0.119	-0.001/0.997	0.036/0.842
PHE	-0.078/0.668	0.138/0.444	-0.361*/0.039	-0.078/0.668	0.135/0.455
THR	-0.085/0.639	0.135/0.455	-0.361*/0.039	-0.085/0.639	0.139/0.440
VAL	-0.039/0.827	0.166/0.355	-0.332/0.059	-0.039/0.827	0.114/0.529
ASP	-0.033/0.854	0.148/0.412	-0.339/0.054	-0.033/0.854	0.095/0.599
SER	-0.032/0.858	0.097/0.592	-0.311/0.078	-0.032/0.858	0.075/0.679
GLU	-0.006/0.974	0.103/0.568	-0.302/0.088	-0.006/0.974	0.051/0.778
GLY	-0.071/0.697	0.139/0.439	-0.391*/0.025	-0.071/0.697	0.128/0.479
ALA	-0.140/0.438	0.128/0.479	-0.373*/0.033	-0.140/0.438	0.186/0.300
TYR	0.039/0.831	-0.015/0.936	-0.281/0.113	0.039/0.831	-0.046/0.801
HIS	0.183/0.308	-0.057/0.753	0.009/0.958	0.183/0.308	-0.187/0.298
ARG	-0.040/0.824	0.126/0.485	-0.326/0.064	-0.040/0.824	0.091/0.614
PRO	-0.179/0.318	0.188/0.295	-0.353*/0.044	-0.179/0.044	0.251/0.159

*Significant ($p < 0.05$).

changes of chlorophyll content between dehydration and rewetting, Fe contents vary much more significant than other minerals. Among all the amino acids detected in this study, proline had the highest coefficient of variation. That may be because proline acts as an important osmotic adjustment substance in physiological adaptation to different environments.

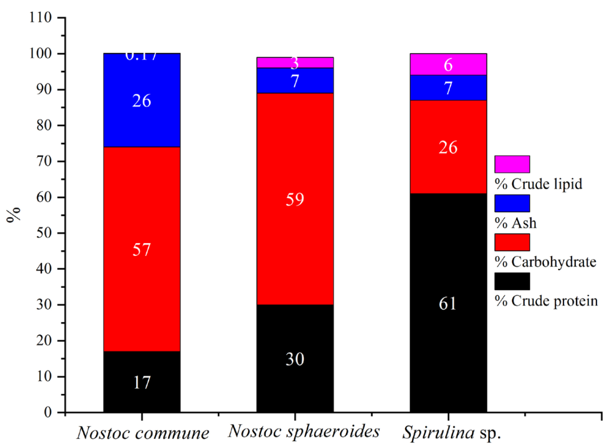

Figure 1. The nutritional contents of *Nostoc commume*, *Nostoc sphaeroides* and *Spirulina sp*.

3.2 The comparison with other algae nutrition

The fat and the carbohydrate are the important components of organisms, which can provide the main energy of the organisms. The crude fat content of *N. commune* was 0.17%,much less than *Nostoc Sphaeroides* and *Spirulina.sp*(Figure 2a). It have been preliminarily confirmed that high-fat diet may be one of the key factors causing depression-like behavior (Hua et al., 2022), *N. commune* may be more suitable for human's diet. Otherwise,the carbohydrate content of *N. commune* was 57%, and the carbohydrate content of *Nostoc Sphaeroides* was 59%, both of *N.commune* and *Nostoc Sphaeroides* contains 2 times as much carbohydrate as *Spirulina.sp* (Figure 2b). Dietary fiber intake provides many health benefits. According to Figure 2c, *N. commune* contains almost 10 times as much dietary fiber as *Spirulina* and three times as much dietary fiber as wheat. High levels of dietary fiber intake are associated with significantly lower prevalence rates for coronary heart disease, stroke, and peripheral vascular disease; major risk factors, such as hypertension, diabetes, obesity, and dyslipidemia, are also less common in individuals with the highest levels of fiber consumption (Wright et al., 2001). High content of dietary fiber makes *N. commune* a potential health food.

The contents of ash in *N. commune, Nostoc sphaeroides* and *spirulina.sp* were 26%, 7% and 7%, respectively(Figure 2d). From a nutritional perspective, *spirulina.sp* contains 61% protein with high nutritional value. *N. commune* contains higher ash and

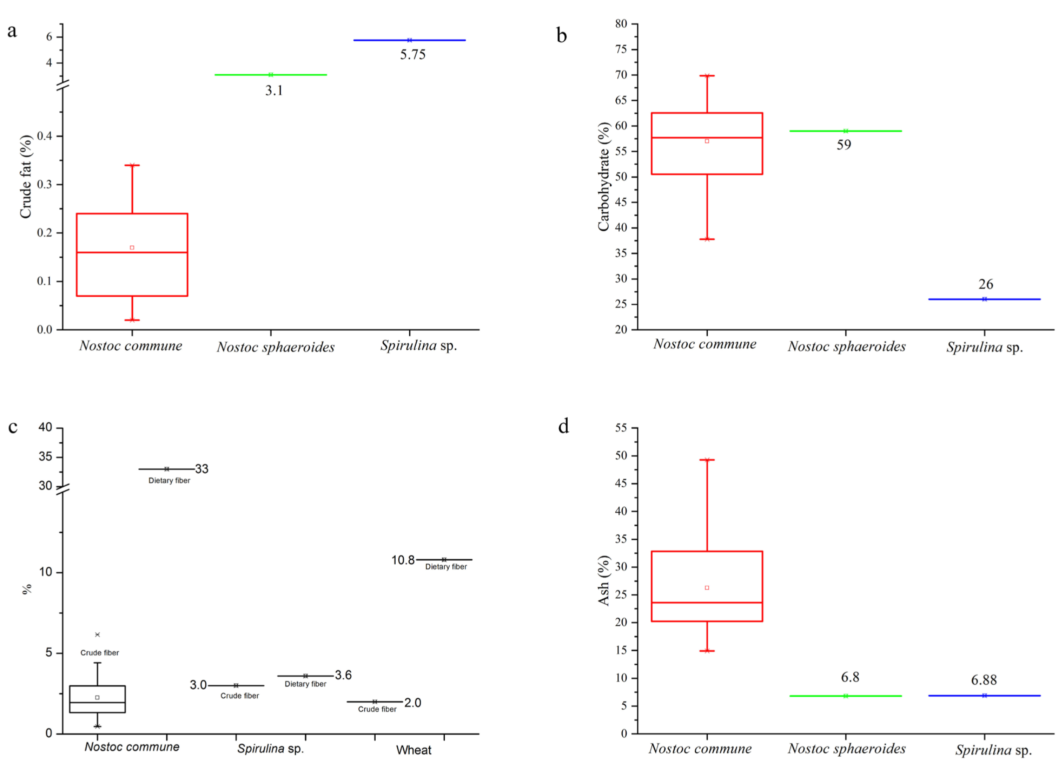

Figure 2. The nutritional contents of *Nostoc commume*, *Nostoc sphaeroides,Spirulina sp* and wheat (a: The crude fat contents of *N.commune, Nostoc sphaeroides* and *Spirulina sp*; b: The carbohydrate contents of *Nostoc commume*, *Nostoc sphaeroides* and *Spirulina sp*; c: The dietary fiber contents of *Nostoc commume*, *Spirulina sp* and wheat; d: The ash contents of *Nostoc commume*, *Nostoc sphaeroides* and *Spirulina sp*).

carbohydrate, as a food, it can timely supply energy and metal elements nutrition.

3.3 Heavy metal contents of N. commune in China

Among five heavy metals tested in this study, Pb, Cr and As contents in all the samples exceeded the Chinese national standard for food (GB 2762-2005, 2012: Pb, 1 mg kg^{-1}; Cr, 2 mg kg^{-1}; As, 0.5 mg kg^{-1}). The most As polluted sample had 16 times of As content as Chinese standard,the most lead polluted sample had 37.59 mg kg^{-1} lead, which was as 37 times of lead content as Chinese standard (Table 1). Arsenic content in Northeast and Northwest of China is relatively high, but the samples with high arsenic content are not only concentrated in Northeast and Northwest provinces of China, but evenly distributed in the whole sample area (Figure S2), so it is possible that *N. commune* had the ability of arsenic enrichment. Lead is a toxic element that is environmentally common and naturally present in soil but accumulates in the body and has serious toxic effects (Onac et al., 2022). Normally lead is not desired in the food. The samples with higher lead content were distributed more in Gansu Province, Shandong Province and Northeast China, while the samples from the south of Yangtze River in China contained less lead (Figure S3). So, it can be concluded that the lead content in *N.commune* samples is directly proportional to the lead content of the geographic sites. The wild resources are not safe enough for eating as food except their heavy metals are removed by some biochemical methods, or wild resources are replaced by biomass of artificial cultivation.

3.4 Geographic variations of biochemical composition and heavy metal content of N.commune in China

There were correlations between chemical compositions and heavy metals with longitude and altitude (Table 4). However, significant negative correlation was found between carbohydrate content and latitude (r = - 0.37, p = 0.05) and a slight positive correlation was found between ash content and latitude (r = 0.36, p = 0.05). A few minerals, such as Cu (r = 0.49, p = 0.01) and Co (r = 0.60, p = 0.00) had significant positive correlations with latitude. Heavy metal Pb also had a significant positive correlation with latitude (r = 0.36, p = 0.04) (Table 4).

According to the above results we could conclude that geographic variations occurred in biochemical compositions, and heavy metals. There was a potential latitude gradient for some chemical compositions, a few minerals and some heavy metals. That is to say, field samples collected from northern areas usually had higher ash, minerals than those from southern areas. Samples from northern areas are more likely polluted by heavy metals.

Ash content of our samples is much higher than that reported in Japan and Philipine (Briones et al., 1997), also higher than artificial cultured biomass (Yan et al., 2010). Besides, there is small variation between samples in the ratio of protein to carbohydrate. Hence, we suggested that ash variation might lead to the variations of protein and carbohydrate. First of all, we exclude that higher ash content came from silt or clay particles adhered to the *N. commune* colonies, because Nostoc colonies were carefully cleared by distilled water in order to remove the soil particles. In fact, soils in the south China have more clay and silt components. If field samples were not washed cleanly, samples from southern China rather than northern China would have higher ash content. Some ash is composed of minerals required for normal growth and metabolism, and the other is composed of minerals or heavy metals that are extra absorbed. Extra absorbed minerals and heavy metals may mainly contribute to the variation of the ash contents.

There are two reasons that might explain the latitude gradients of ash, minerals and heavy metals. One of them is that samples came from lower latitudes usually have longer wetting time and more suitable temperatures, so they grow faster than those from higher latitudes. Faster growth dilutes the content of ash. The other reason is that lower latitude areas have more precipitation and the rainfall continuously washes away the minerals and heavy metals adsorbed. That not all the minerals and heavy metals have latitude gradient may be caused by interact effects of minerals and heavy metals such as synergy absorption and antagonism absorption.

Compared our results with reports in Russia, China samples had more heavy metals (Patova et al., 2000; Patova & Sivkov, 2003). This result is in accordance with the regional pollution characteristics. More interesting, geographic gradients of heavy metals in *N.commune* samples are contradict with those in soils, because soils in southern China were more seriously polluted by heavy metals than soils in northern China according to the soil pollution condition investigation communiqué (Chen et al., 2015). It can be referred that most of the minerals and heavy metals absorbed by *N.commune* mainly come from rainfall or runoff created by rainfall. *N.commune* seldom absorbs minerals and heavy metals directly from soils. This can be confirmed by the observation of the habitat of *N.commune*. Sometimes, they grow very well on the rock surfaces or in the interfaces of moss stems and leaves and no direct contact were found between Nostoc colonies and soils.

Table 4. Correlation coefficients between biochemical compositions and heavy metals with geographic variables of *N. commune*.

Correlation coefficients	Longitude	Latitude	Altitude
Protein (n = 32)	0.17/0.34	-0.11/0.55	-0.28/0.12
Lipid (n = 32)	0.06/0.74	-0.16/0.39	0.15/0.40
Ash (n = 30)	-0.31/0.10	0.36/0.05	0.29/0.12
Carbohydrate (n = 30)	0.28/0.13	-0.37*/0.05	-0.26/0.16
Protein/Carbohydrate (n = 30)	0.14/0.46	0.28/0.13	-0.07/0.70
Fe (n = 33)	-0.02/0.91	-0.30/0.09	-0.08/0.66
Ca (n = 33)	0.17/0.33	0.04/0.85	-0.18/0.31
Cu (n = 33)	-0.07/0.69	0.49**/0.01	0.01/0.94
Ze (n = 33)	0.01/0.95	0.28/0.11	-0.06/0.76
Mn (n = 33)	-0.14/0.45	0.26/0.14	0.02/0.90
Co (n = 33)	-0.26/0.14	0.60**/0.00	0.23/0.19
Se (n = 33)	0.13/0.47	0.14/0.43	0.15/0.41
Pb (n = 33)	0.04/0.83	0.36*/0.04	-0.04/0.82
Cd (n = 33)	0.03/0.89	0.20/0.27	-0.06/0.76
Cr (n = 33)	-0.21/0.23	0.20/0.27	0.18/0.32
Hg (n = 33)	-0.07/0.68	-0.14/0.45	0.12/0.50
As (n = 33)	-0.22/0.23	0.28/0.12	0.19/0.29

*Significant ($p < 0.05$). **Significant ($p < 0.01$).

4 Conclusions

The dry mass of *N.commune* is mainly composed of carbonate rather than protein. Most of the carbonate was dietary fibre. Meanwhile, it has a higher content of Ca and Fe minerals. Samples from different areas may have different nutrient compositions and different safety. There were significant features in the nutrient composition and safety with latitude in China. With the increasing latitude, carbonate content tended to decrease and safety tended to increase. In all,if the heavy metals Pb, Cr and As are removed,*N.commune* can be good resources of carbonate and protein to human being and animals.

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Supplementary material

Supplementary material accompanies this paper.

Figure S1. Sampling locations of N. commune in China.

Figure S2. The As content distribution of China and the sampling location (based on As content) of China.

Figure S3. The Pb content distribution of China and the sampling location (based on Pb content) of China.

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