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Determination of carbon, nitrogen and phosphorus in cattail using cold neutron prompt-gamma activation analysis

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A method for the determination of carbon, nitrogen, and phosphorus in cattail using cold neutron prompt-gamma activation analysis (CNPGAA) has been developed and evaluated through the analysis of standard reference materials (SRM). After extensive preparation, approximately 400 mg cattail samples from the lower Apalachicola River floodplain were irradiated in the CNPGAA facility at the National Institute of Standards and Technology (NIST). The results of numerous field samples and two standard reference materials using the nuclear method show favorable comparison to results obtained by a CHNS/O analyzer.

Introduction

Over the years, various plant species, including cattails, have been identified as biomonitors for nutrients and heavy metals.[1,2] Cattails usually grow on the margins of lakes and riverbanks, in ponds, marshes and meadow bogs. Although this plant is indigenous to these ecosystems throughout the United States, nutrient enrichment in sediment and the water column can lead to undesirable expansion.[3] Several studies[4,5] in the Everglades have demonstrated that cattails are dominant in areas of phosphorus enrichment. Phosphorus enrichment in sediment and water is a major driving force of cattail expansion. Early signs of this expansion are apparent in the lower Apalachicola River floodplain near the city of Apalachicola located in northwest Florida, USA. The overall goal of this research project is to use cattails as biomonitors of nutrient enrichment in the lower Apalachicola River floodplain.

In order to meet the overall goal of this project, it is necessary to analyze a large number of samples collected from eight stations in the study area during wet, growing, and dry seasons. Typically, C, N, and P in cattails are determined by traditional chemical methods such as a CHNS/O elemental analyzer (EA) for C and N, or the colorimetric method for P. However, traditional chemical methods are generally labor intensive and often require sample dissolution via acid digestion. CNPGAA allows nondestructive, rapid, and simultaneous multielement analysis of C, N, and P as well as other light elements (H, B, Si, S, Cl) that cannot be easily measured by other methods.[6,7]

In the initial phase of this project it was necessary to develop the details of the CNPGAA method and evaluate it through the analysis of standard reference materials (SRM). The results of field samples and two SRMs using the nuclear analytical method are compared to those obtained by a CHNS/O elemental analyzer. A comparison of plant data is presented.

Experimental

Study area

The Apalachicola National Estuarine Research Reserve, the largest of 25 National Estuarine Research Reserve sites, consists of two barrier islands (St. Vincent Island and St. George Island), the lower 84 kilometers of the Apalachicola River and its associated floodplain, portions of adjoining uplands, and the Apalachicola Bay.[8]

There are eight stations in the study area located in the lower Apalachicola River floodplain. Each station includes three sampling locations separated by about 10 m. Cattail coverage occurs in dense non-contiguous patches along the Apalachicola River between Stations 1 and 5. Station 8, located in the little St. Mark River, approximately 1.5 km away from the Apalachicola River, serves as a reference area.

Sample collection and preparation

To investigate the biomass and nutrient in cattails in the lower Apalachicola River floodplain, cattails were collected from Stations 1, 5, 7, and 8 during September 2002. Taking care to keep plants attached by rhizomes, cattails in an area of 0.5 m×0.5 m were removed. Cattails were stored in coolers at 4 °C for transportation to the laboratory.

In the laboratory, cattails were separated into four parts based upon the tissue function, i.e., shoot/carbon fixation, root/nutrient uptake, rhizome/vegetation growth, and shoot base/storage. Cattail samples were washed with tap and distilled water; dried to constant mass in an oven (60 °C),[2] and ground to a fine powder (<200 mesh) by a blender for analysis.[9]

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CNPGAA procedure

Prompt gamma-ray activation analysis using thermal neutrons (TNPGAA) has been used for the determination of light elements in biological materials.[10–13] Although PGAA instruments using guided beams of cold (low-energy) neutrons offer the advantage of better detection limits than thermal neutron instruments for most elements,[6] CNPGAA has not been used widely for the analysis of biological materials because of the effects of neutron scattering by hydrogen in the sample. Element sensitivity (in counts·g^{-1}·s^{-1}) for a particular analytical system is a function of a neutron capture cross section and the shape and size of samples.[14] Neutron scattering by hydrogen ($\sigma_s = 80$ barns, σ_s: bound-atom scattering cross section) affects the neutron capture cross section by altering the average distance the neutron travels in a sample. Monte Carlo simulations have shown that elastic neutron scattering, without changing the neutron energy, can change element sensitivity.[15] Neutron scattering may either increase or decrease element sensitivity since a scattered neutron may be absorbed in the sample, scattered out of the sample, or scattered multiple times in the sample.[16] While MACKEY et al.'s experiments[17] show that element sensitivity increases with increasing hydrogen content in the sample in thermal neutron prompt-gamma activation analysis (TNPGAA), PAUL et al.'s experiments[14] show that element sensitivity decreases with increasing hydrogen content in CNPGAA. These two cases can be explained as follows: First, the scattering cross section of H for cold neutrons is larger than that for thermal neutrons. Depending on the sample shape, this may result in more cold neutrons escaping out of the sample so that the average number of cold neutrons in the sample is lower than thermal neutrons. Second, cold neutrons unlike thermal neutrons are not in thermal equilibrium with a sample at room temperature so that the scattering results in an increase in the average energy of the cold neutron beam. This decreases the effective absorption cross section which results in a decrease in element sensitivity.[14]

Previous studies[14,17] have also shown that element sensitivity can be affected by the thickness of samples as a consequence of neutron self shielding. However, results from CNPGAA can be made more accurate by matching the sample thickness and hydrogen content between samples and standards.[18–20]

Sample and standard preparation: Three groups of standards for C, H, N, and P were prepared from mixtures of monomethyl phosphate di-cyclohexylammonium salt ($CH_5O_4P{\cdot}2C_6H_{13}N$), cellulose ($C_6H_{10}O_5$), silicon dioxide ($SiO_2$), graphite (C), and mannitol ($C_6H_8(OH)_6$) (Table 1). To keep the range of C, N, H, and P in standards consistent with those found in cattails (C: 42.94%, 41.97 to 43.91%; N: 0.91%, 0.58 to 1.41%, and H: 5.71%, 5.38 to 5.86%),[21] the different mixtures were prepared with the five chemicals above combined in different ratios. Mixtures were prepared using a mixer mill. In order to investigate how element sensitivities of C, N, and P vary with changes in hydrogen content in the sample, the first group of standards was prepared by varying the hydrogen mass fraction from 3.13% to 10.24% while keeping the same thickness of disks (2.7 mm) as cattails (std-1, 2 and 3 in Table 1). To understand how the element sensitivity of C, N, and P varies with changes in the thickness of disks, the second group of standards was prepared by varying the thickness of disks from 1.5 mm to 4.1 mm while keeping the same hydrogen content ($m_H = 22.84$ mg) as cattails. For calibration purposes, a third group of standards (std-2, 5, and 7 in Table 1) was prepared by matching the thickness of disks (2.7 mm) and hydrogen content (22.84 mg) of calibration standards to that of cattails while also maintaining C, N, and P concentrations around the above range of values for C, N, and P found in cattails in the lower Apalachicola River floodplain. Standard composition details are provided in Table 1.

Cattails were prepared into above ground (shoot) and below ground sections (shoot base, root, and rhizome) for CNPGAA. After drying in a desiccator at room temperature for 120 hours over fresh anhydrous magnesium perchlorate (the sample depth <1 cm),[9] approximately 400 milligrams of powdered cattails, or standard reference materials were pressed into pellets (approximately 12.7 mm diameter, 2.7 mm thickness) using a stainless steel die and hydraulic press. The standards were also pressed in the same manner. In order to prevent contamination between samples, the die was thoroughly cleaned with deionized water and ethanol. Pellets were sealed in Teflon bags.

Sample irradiation: The bag along with the pellet was mounted by suspension between Teflon strings tied on the prongs of an aluminum fork. This assembly was then placed into an evacuated magnesium sample chamber (evacuated to eliminate background from nitrogen in the air). Samples were irradiated for about 7 hours in the CNPGAA facility (thermal equivalent neutron flux of $9{\cdot}10^8$ n·cm^{-2}·s^{-1}) at the 20 MW research reactor at the NIST Center for Neutron Research in Gaithersburg, MD in USA. An empty Teflon bag was irradiated for 7 hours in order to determine the C, N, H, and P blank.

Data collection and processing: Compton suppressed 16K spectra up to 11 MeV were collected using a high purity germanium (HPGe) detector, an Acquisition Interface Module (AIM), and a digital signal processor operated under the control of a Canberra Genie Workstation based on a DEC VAX station. A titanium foil was irradiated before and after irradiating a sample to monitor changes in neutron fluence rates.

Table 1. Standards information

Standard identity/composition	H mass fraction, %	Standard mass, mg	H mass, mg	Disk thickness, mm
Std-1 8% A + 30% B + 62% C	3.13	478.43	14.90	2.7
Std-2 5.5% A + 54.5% B + 40% C	4.75	475.67	22.62	2.7
Std-3 A	10.24	399.71	40.93	2.7
Std-4 A	10.24	223.88	22.93	1.5
Std-5 17% A + 30% B + 36.5% C + 9% D + 7.5% E	4.51	499.17	22.52	2.7
Std-6 8% A + 30% B + 62% C	3.13	724.00	22.63	4.1
Std-7 8.2% A + 34.9% B + 48.23% C + 8.61% D	4.55	497.02	22.62	2.7
Std-8 5.5% A + 54.5% B + 40% C	4.75	739.80	35.17	4.1
Std-9 A	10.24	615.99	63.07	4.1

A: $CH_5O_4P{\cdot}2C_6H_{13}N$.
B: $C_6H_8(OH)_6$.
C: SiO_2.
D: C (graphite).
E: $C_6H_{10}O_5$.

The count rates of C, H, N, and P were normalized to the average Ti flux monitor count rate measured during the analysis of all samples.

In CNPGAA, concentration is usually calculated by matching the H content and disk thickness at the same time between standards and samples.[18,19] However, it is very difficult to match these quantities during sample preparation in practice, especially for field samples in which the H content varies. Therefore, a new method was proposed in the present study that simulates a sensitivity curve using a group of standards with different thickness of disks and H content. According to our previous work, it was found that near the H concentration of field samples, element sensitivities of C, N, and P vary proportionally with H content and thickness of disks. It was assumed, therefore, that Eq. (1) could be applied:

$$S = aX + bY + c \quad (1)$$

where S is the sensitivity, X is the H content, and Y is the thickness of a disk.

The constants a, b and c can be obtained by a least squares fit from a group of standards with different H content and thickness of disks. During calculations of sample concentrations, the H content was first calculated using the average sensitivity determined from the third group of standards, then by using a known thickness of disks and H content, the sensitivities of C, N, and P were calculated using Eq. (1).

Spectra were transformed as a text file using the Fullist code developed at NIST.[22] A Microsoft Excel spreadsheet was used for fitting calibration curves and subsequent calculations of peak area integration,[23] concentration, uncertainty, neutron fluence rates, pileup and background corrections, etc.

Although samples were dried to constant mass prior to irradiation, samples lost weight (<3%)[21] during the 7-hour evacuated irradiation so that the hydrogen content in the sample lost about 0.3% during the irradiation. Experimental data also show that the count rate for H was similar before and after the irradiation. Thus, the count rate for H during the irradiation and the mass obtained after irradiation were used in the calculations for H, C, N, and P.

Results and discussion

As can be seen in Fig. 1, the sensitivities of C, N, and P decrease as H content increases. The H effect on the sensitivity of P is larger than that for C and N with N being the least affected. Figure 1 also shows that the sensitivities of C, N, and P decrease as the thickness of disks increases. The thickness of disks effect on the sensitivity of P is also larger than that for C and N.

Table 2. The results of SRM from CNPGAA and EA

Sample	P mass fraction, %			C mass fraction, %				N mass fraction, %				H mass fraction, %		
	CNPGAA			CNPGAA				CNPGAA				CNPGAA		
	Average sensitivity	Fit curve	Certified value	Average sensitivity	Fit curve	EA	Certified value	Average sensitivity	Fit curve	EA	Certified value	Average sensitivity	EA	Certified value
SRM1570a	0.550	0.524		38.6	41.6			6.29	5.94			5.67		
Spinach Leaves	0.530	0.502		38.2	40.7			6.26	5.87			5.43		
Mean:	0.540	0.513	0.518 ± 0.011	38.4	41.2	41.3 ± 0.1	40.1 ± 0.2[+]	6.28	5.91	6.00 ± 0.01	6.06 ± 0.20	5.55	5.70 ± 0.08	5.60 ± 0.17[+]
σ:	0.014	0.015		0.3	0.6			0.02	0.05			0.16		
RSD, %	2.593	2.924		0.8	1.5			0.32	0.85			2.88		
SRM1573a	0.244	0.230		37.1	39.2			3.24	3.03	#		5.24		
Tomato Leaves	0.230	0.213		39.4	41.9			3.26	3.06	#		5.33		
Mean:	0.237	0.222	0.216 ± 0.004	38.3	40.6			3.25	3.05	3.03 ± 0.15		5.28		
σ:	0.010	0.012		1.6	1.9			0.01	0.02			0.07		
RSD, %	4.219	5.405		4.2	4.7			0.31	0.66			1.33		

σ: Standard deviation.
RSD, %: Relative standard deviation.
[#] RSM 1573a used in EA for the calibration curve.
[+] Reference from D. L. ANDERSON's (NIST) paper.

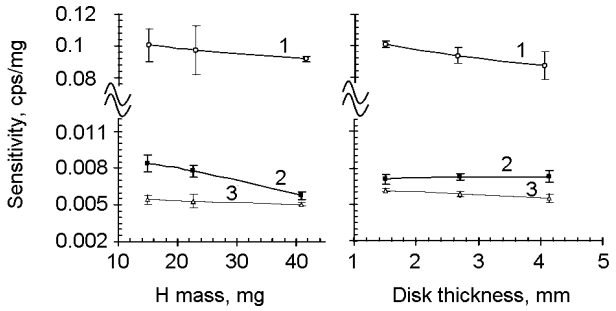

Fig. 1. H and disk thickness effect on the sensitivities of C (1), N (2) and P (3). Uncertainty at 95% confidence level

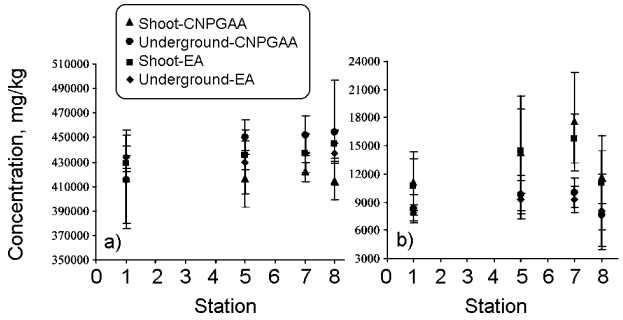

Fig. 2. C (a) and N (b) in the shoots and undergrounds. Mean: average value from trip sites in each station ($n = 3$); uncertainty at 95% confidence level

The calibration curves of C, N, and P were simulated by Eq. (1) using the 9 standards in Table 1. The values for these curves of C, N, and P as donated as S_P, S_C, and S_N, respectively, are:

$$S_P = -0.000299\ X - 0.005853\ Y + 0.118862$$
$$S_C = -0.000070\ X - 0.000037\ Y + 0.008559$$
$$S_N = -0.000033\ X - 0.000030\ Y + 0.006971$$

To evaluate the accuracy of these calibrations, elemental concentrations of C, N, H, and P in SRM1570a trace elements in Spinach Leaves and SRM1573a Tomato Leaves were calculated using the average sensitivities calculated by std-2, 5, and 7 in Table 1 and the above calibration curves and compared to the results of C, N, and H obtained by a CHNS/O elemental analyzer (EA) (Table 2). As shown in Table 2, the results of P show that the difference between the results obtained by the calibration curve and the certified value is less than 3% while the difference between the results obtained by the average sensitivity and the certified value is less than 10%. The difference for C between results obtained by the calibration curve and the certified value is about 2% while the difference between results obtained using the average sensitivity and the certified value is about 7%. The difference for C between results obtained from an EA and the certified value is about 3%. For H, the three methods (calibration curve, the average sensitivity, and EA) obtained essentially the same results as the certified value. The difference for N between results obtained by the calibration curve and the certified value is about 3% while the difference between results obtained by the average sensitivity and the certified value is about 4%, and the difference between the results obtained from an EA and the certified value is about 1%. Therefore, the results from the three methods are comparable. The calibration curve method yields better agreement with the certified values than the average sensitivity, especially for P and C.

Concentrations of C and N in cattails were calculated by the calibration curve and compared to the results of obtained from an EA (Fig. 2). The results show that the C difference between the CNPGAA and EA method is less than 7%, and the N difference between the CNPGAA and EA method is less than 11%.

To understand the growth of cattails in this ecosystem, cattails will be collected from 8 stations and analyzed during wet, growing, and dry seasons in the future.

Conclusions

A comparison of analytical results for a large amount of cattails and two SRMs obtained using CNPGAA and an EA shows that CNPGAA is a reliable alternative to

the traditional chemical method. The CNPGAA method affords a simultaneous multielement analysis of C, N, and P in cattails. In large field studies, CNPGAA can also result in decreased cost and time. This is an example of how to correct for the effects of H content and thickness of samples in the analysis of biological materials.

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