Advances in Polar Science

doi: 10.3724/SP.J.1085.2011.00092 June 2011 Vol.22 No.2: 92–100

Alkaline phosphatase activity in ornithogenic soils in polar tundra

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Received Apricl 10, 2011; accepted June 13, 2011

Abstract Phosphatase plays an important role in the microbial liberation of phosphorus in soil systems. In this study, alkaline phosphatase activity (APA) was investigated from tundra ornithogenic soil profiles in Antarctica and Arctic. The organic carbon (TOC), total nitrogen (TN), and phosphorus fractions and pH were also analyzed in these soils. The correlation between APA and soil chemical properties is discussed. In almost all the soil profiles, APA showed the same variation patterns. The maximum APA appeared in the surface layers, and decreased with soil depth. The APA ranged from 1.00 ppm to 1 403.5 ppm with an average of approximately 408.3 ppm. The APA showed a significant positive correlation with TOC (r =0.70, p <0.001), TN (r =0.43, p =0.002), total phosphorus (r =0.39, p <0.05)and inorganic phosphorus (r =0.40, p =0.037), indicating that they were predominant factors affecting APA in the polar tundra soils. In addition, APA showed a significant negative correlation with Cu and Zn concentrations in the soils, indicating that Cu and Zn may inhibit APA. Our results showed that APA could be used as an important indicator for soil fertility in polar tundra ecosystems.

Keywords **Tundra ornithogenic soil, alkaline phosphatase, organic carbon, total nitrogen, phosphorus**

Citation: Ma D W, Zhu R B, Ding W, et al. Alkaline phosphatase activity in ornithogenic soils in polar tundra. Adv Polar Sci, 2011, 22: 92—100, doi:10.3724/SP.J.1085.2011.00092

0 Introduction

Phosphatase, as an important enzyme for regulating phosphorus cycling and it is one type of enzyme catalyzing the hydrolysis of organic esters and anhydrides[1-2]. Phosphatase activity directly affects the decomposition and transformation of soil organic phosphorus and its bioavailability[3]. Therefore, phosphatase plays an important role in the biological liberation of phosphorus in soil systems[4-5].

Phosphatases are secreted into the soils by plant roots and soil microorganisms, only the small fractions are released into the soil solution and most of them are absorbed onto the soil colloids and clay minerals[6]. According to pH values, phosphatases can be divided into acid phosphatase, neutral phosphatase and alkaline phosphatase. Acid soils are dominated by acid phosphatase while calcareous soils are dominated by alkaline and neutral phosphatases due to the formation of a large amount of calcium carbonate via alkaline reactions in the soils[3]. Soil pH has an important effect on the distribution, activity and stability of phosphatase. It has been found that H^+ concentration was one of the predominant factors influencing phosphatase in wetlands, and waterlogged conditions and low temperature affects its activity[7]. Phosphatase activity is also affected by soil carbon, nitrogen and organic matter content, thus it can be used as an indicator for soil fertility[6]. It has been reported that there has been obvious differences in APA activity with depth in meadow soils, and the highest APA occurred in the surface layers and the lowest at the bottom of the profile. Alkaline acid phosphatase enzyme activity has also shown a significant correlation with soil organic matter and total nitrogen content[8]. Physical properties of the soils, such as soil moisture, temperature and aeration, affect the microbial population releasing all kinds of enzymes, and thus impact on soil APA. Long-term increases in soil water pressure can led to reduced APA[9]. Phosphatase has also shown a positive correlation with soil oxygen content[10]. In addition, the different ways soils are managed, such as type of tillage and crop rotation employed[11], use of herbicides, pesti-

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cides[12] and heavy metals[13-19] can also affect soil phosphatase activity.

In summary, the phosphatases have been extensively studied in different soils at the global scale[7-24]. However, phosphatase activity and its regulating factors have seldom been studied in polar tundra soils. Although most polar soils may be very barren due to weak weathering and the absence of vegetation, the soils around sea animal colonies are strongly impacted by animal excreta, leading to the formation of fertile ornithogenic soils rich in organic carbon (OC), nitrogen (N) and phosphorus (P). Phosphatase plays an important role in the transformation of P, therefore, it is important to conduct an investigation into phosphatase activity in polar soils.

In this study, the vertical distribution characteristics of APA were investigated in polar tundra soils or sediments, and organic carbon, nitrogen and phosphorus concentrations and the pH values were determined in the soils or sediments. The correlations were analyzed between phosphatase activity and environmental factors, and the factors affecting phosphatase activity are also discussed in relation to polar ornithogenic soils/sediments.

1 Materials and methods

1.1 Study area

The first study location was the Vestfold Hills, which are E) in the Ingrid Christensen Coast of Princess Elizabeth Land (Figure 1). It consists of the areas known as Long Peninsula, Broad Peninsula and Mule Peninsula with an ice free area of about 400 km^2[25]. This area has a characteristic continental Antarctic climate. Temperatures are usually below 0℃ for most of the year, with summer air temperatures rising as high as 9℃ and winter temperatures dropping to as low as －40℃[26]. In the summer of 2005/2006, we investigated penguin colonies on Long Peninsula Island, Magnetic Island, Gardner Island and Zolotov Island in the Vestfold Hills, and collected four ornithogenic sediment cores named as DAP, DM1, DG2 and ZOL2.

The second study area was the Millor Peninsula (69°23' S– 69'°56'S, 76°20' E –76°45'E), which is located in coastal East Antarctica with an ice-free area of about 40 km^2. This area is also characterized by a typical cold, dry continental climate[25, 27]. According to meteorological data collected at ZhongShan Station, the average temperature of this area is about －10℃, the annual precipitation is about 250 mm and the annual average relative humidity is about 60%, There is no true soil development due to extremely cold weather conditions, exposed bedrock and the rare vegetation[25]. In the summer of 2005/2006, emperor penguin guano samples (named as DQ) were sampled from an emperor colony in Amanda Bay and one sediment core (named as ZH3) was also collected from Lake Mochou on Millor Peninsula (Figure 1).

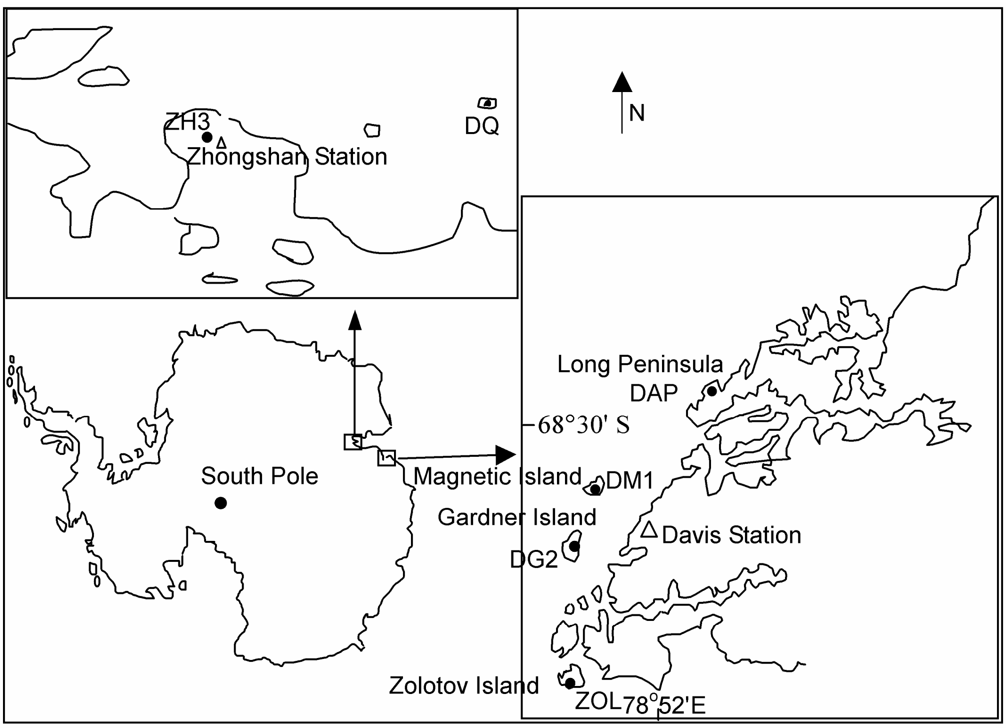


Figure 1 The sampling sites for the ornithogenic soils/sediments impacted by penguin guano in Antarctica. Note: ZH3 is the site for Lake Mochou sediment; DQ is the site for emperor penguin guano in Amanda Bay; DAP is the site for ornithogenic soils on Long Peninsula; DM1 is the site for ornithogenic soils on Magnetic Island; DG2 is the site for ornithogenic soils on Gardner Island and ZOL is the site for the ornithogenic soils on Zolotov Island.

The third study area was the Ny-Alesund area (78°55' N, 11°56' E) on the Arctic Spitsbergen Island (Figure 2). It is an important expedition base in the Arctic region. Carboniferous-Permian limestone and dolomite are the main rock types here, forming a solid and stable basic rock. It is the coldest in February with an average temperature of about －14℃ and in July the warmest, with an average temperature of about 5℃. The annual average temperature is approximately －5.8℃, and the annual average precipitation is about 400 mm[28]. During the Arctic scientific expedition in summer of 2007, one sediment core (named as L1) was collected from a lake before the Chinese Arctic Yellow River Station. One ornithogenic soil profile (named as ND) was also collected from Bird Island (Figure 2).

1.2 Sampling description

The sediment cores ZH_3, DG2 L1 and ND were collected using clean PVC pipe with a 12 cm diameter and their lengths were about 80 cm, 68 cm, 40 cm and 45 cm, re--specttively; The ornithogenic profiles DAP, DM1, ZOL and DQ were sectioned in situ at 1cm intervals from top to bottom with a bamboo scoop, respectively. All the samples were bagged and taken back to the laboratory for description of the lithology. The sediment cores were sectioned at 1 cm intervals and preserved in a －10℃ freezer prior to study.

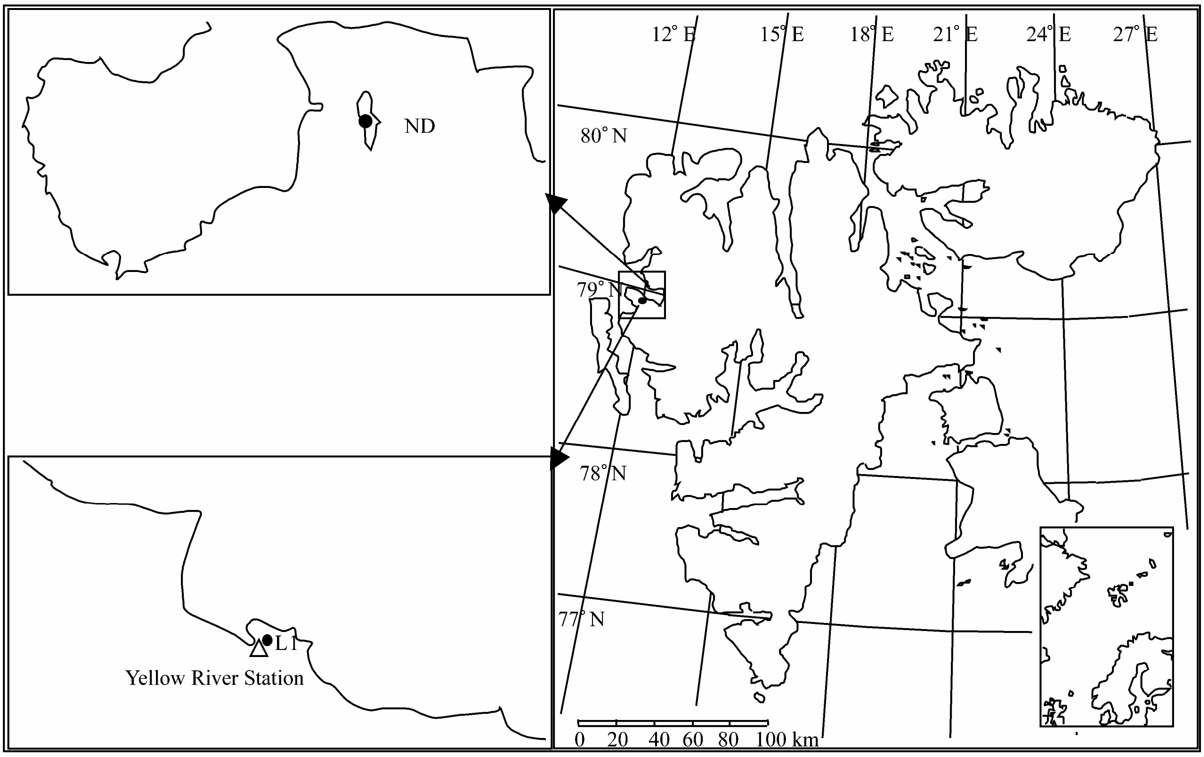


Figure 2 The sampling sites for the ornithogenic soils/sediments in the Arctic. L1: The site for the sediment taken from the lake near Chinese Yellow River Station and ND the site for the ornithogenic soil on Bird Island.

1.3 Measurement of phosphatase activity

Soil phosphatase activity was determined according to the Hofmann method[29]. Phosphatase is a hydrolysis enzyme catalyzing organic phosphorus compounds. It can hydrolyze organic phosphorus compounds in soils and lead to the release of inorganic phosphorus. The following is the basic reaction:

$$R—O—\underset{\displaystyle ONa}{\overset{\displaystyle ONa}{P}}=O \xrightarrow[H_2O]{\text{Phosphatase}} R—OH+Na_2HPO_4$$

(Where, R refers to phenolphthalein, phenol, glycerol, α-or β-naphthol or p-nitro phenol). The soil phosphatase activity was determined by the measurement of phenol produced during the substrate hydrolysis. This method can be used to measure the activity of various phosphatases by changing the pH value of the buffer solution: acid phosphatase(pH 5.0, acetate buffer solution), neutral phosphatase(pH 7.0, citrate buffer solution), alkaline phosphatase(pH 10.0, borate buffer solution). The detailed processes of the measurements were as follows:

The 2.5g air-dried soil samples were put into a 100 mL volumetric flask and treated with 1.5 mL toluene. After 15 min, 2.5 mL of benzene disodium solution (25 mg/mL) and 2.5 mL borate buffer were added (Note: the ornithogenic soils were alkaline (pH 5.9 - 8.0, Table 1), therefore only alkaline phosphatase activity was determined). The reaction mixture was placed in the incubator at 37℃. After 3 h, 38℃ water was used to dilute the solution, and then filtered with a dense filter. For each soil sample, 2.5 mL water was used as a control instead of a substrate. The 1 mL filtrate was transferred into 100 mL volumetric flasks, and 5 mL borate buffer solution and 1 mL 2, 6-Dibromoquinone-4-chloroimide (2 mg/mL ethanol) reagent were added, and then the reactants were mixed for 20 min until the color of the solution changed to green. The solution was diluted with water, and then measured with a spectrophotometer at 578 nm. The amount of phenol produced was calculated as follows :

Soil phosphatase activity [μg phenol/g dry soil]
= (C × V) / dwt.

Where, C indicates the phenol content of the soil filtrate (μg/mL); V indicates soil solution volume (mL); dwt indicates the dry soil weight (g).

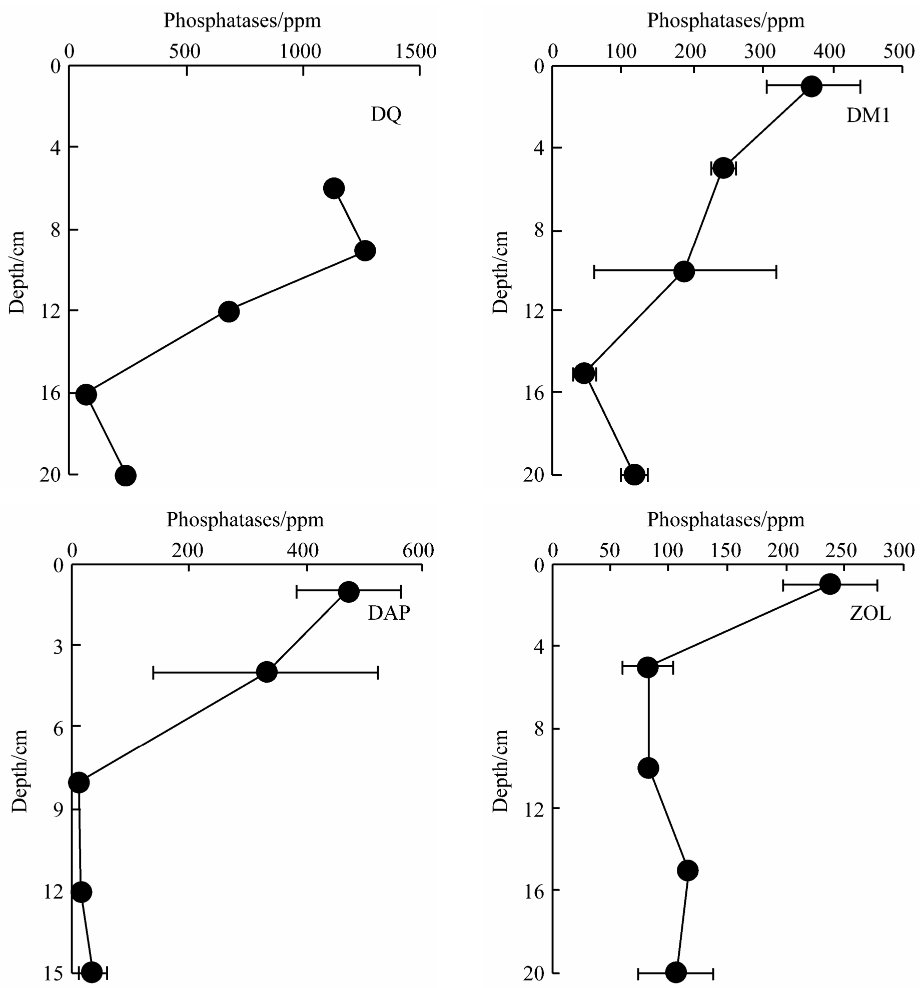
1.4 Analyses of soil chemical properties

Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5. Total nitrogen in the soils were measured through the German Vario.EL element analysis procedure, and the relative error was less than 1%. The organic carbon were measured by the potassium dichromate oxidation-Ferrous sulfate titrimetry. Soil inorganic phosphorus, organic phosphorus and total phosphorus were measured by the ammonium molybdate spectrophotometric method. The samples were digested by using HF-HNO_3-$HClO_4$, and the Cu and Zn concentrations were determined using a Optima 5300 V plasma emission spectrometer, where the relative error was less than 1%.

2 Results and discussion

2.1 Variations of APA in ornithogenic soil/sediment profiles

As shown in Figure 3, alkaline phosphatase activity (APA) showed a different distribution pattern with depth at the three sampling sites. In the profiles DQ, DM1, DAP, ZOL, DG2 and ND, the maximum APA occurred in the surface layers, then APA rapidly decreased and reached almost stable values at the lower depths. In the two ornithogenic lake sediment profiles ZH3 and L1, APA significantly decreased with depth and then increased.



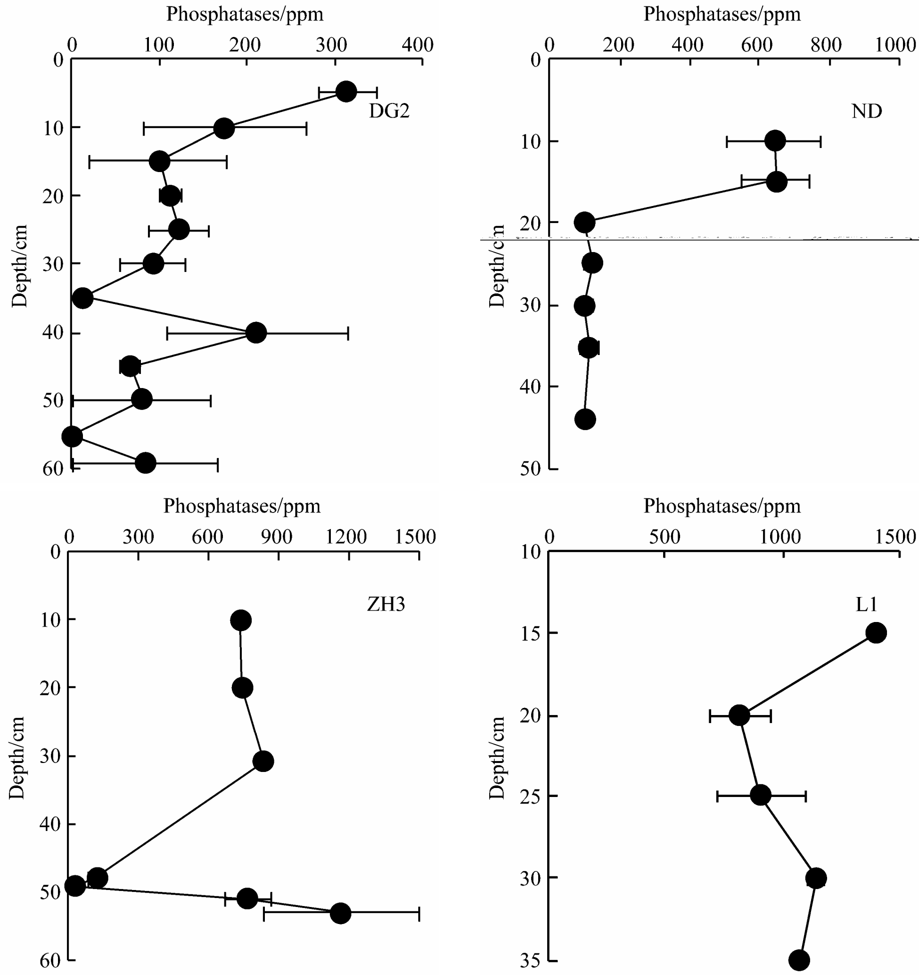


Figure 3 Variations in alkaline phosphatase activity with depth in the ornithogenic profiles (n=2, Error bars represent standard deviation).

For all the soil profiles, the average APA, TP, IP, OP, OC, TN and pH are listed in Table 1. The Antarctic ornithogenic soils and penguin guano were slightly alkaline with a pH range between 7.0 and 8.0, close to those in the Arctic ornithogenic profiles, ND and L1, while the OC contents were less than 4.0%, lower than those in the Arctic profiles. The TN contents ranged from 0.18% to 2.1%. The TP, IP and OP concentrations in the Antarctic ornithogenic soils and penguin guano were one order of magnitude higher than those in lake sediments and Arctic soils, with the highest IP and TP concentrations being in the profiles DM1 and DAP.

In the Arctic orni- thogenic soils and lake sediments, the concentration of the different forms of phosphorus were relatively low (<800 ppm). In addition, the APA was generally lower than 300 ppm in the ornithogenic soil profiles while the APA was considerably higher (>600 ppm) in the emperor penguin guano and lake sediments with the highest APA (1 070.2 ppm) being found in the Arctic lake sediment profile L1.

Table 1 Chemical properties for the different ornithogenic soil or sediment profiles in the polar regions

Type	pH	OC/%	TN/%	IP/ppm	OP/ppm	TP/ppm	APA/ppm
Antarctic							
Ornithogenic soil profile DAP	7.16	2.95	0.95	13 126.01	4 678.56	1 7804.56	172.27
Ornithogenic soil profile DM1	7.24	3.98	2.06	38 411.86	9 344.17	4 7756.03	193.77
Ornithogenic soil profile ZOL	7.35	1.44	0.18	1 517.48	568.41	2 085.89	124.90
ornithogenic soil profile DG2	8.01	3.76	0.27	7 816.10	1 726.13	9 542.23	114.75

(To be continued on the next page)

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Type	pH	OC/%	TN/%	IP/ppm	OP/ppm	TP/ppm	APA/ppm
Emperor penguin guano DQ	6.98	14.2	2.09	5 373.88	446.81	5 820.68	698.83
Lake Mochou sediment core ZH3	5.87	11.3	0.82	442.10	392.36	834.47	630.71
Arctic							
ornithogenic soil profile ND	7.91	13.8	0.73	368.15	376.19	744.34	260.99
Lake sediment core L1	6.60	21.2	1.23	371.88	426.88	798.76	1 070.22

2.2 Relationships between APA and OC, APA and TN

As illustrated in Figure 4, APA showed a significant positive correlation with OC (r = 0.70, p <0.001) and TN (r =0.43, p =0.002) in the ornithogenic soils/sediments, indicating that soil carbon, nitrogen and organic matter content had important effects on APA, which is consistent with previous results[8, 24, 30-31]. The soils or sediments were strongly influenced by penguin guano or seal excrement and they are enriched in OC and N[20], thereby stimulating microbial and soil enzyme activity. In soil systems, the OC and TN contents decrease with depth and soil microbial and enzyme activities also show similar variations, indicating that soil carbon and nitrogen contents are major factors affecting phosphatase activity, and hence it could be used as an indicator of soil fertility[32]. Organic matter increases soil porosity, and improves soil aeration and structure, and thus affects the soil microbial population and its activity, as well as the activity of the soil enzymes[33]. In addition, phosphatase activity has an important effect on the decomposition and transformation of soil organic matter[30]. Other researchers have also shown that APA was closely correlated with OC and TN in soils[8, 21, 31]. Therefore, the polar tundra ornithogenic soils are a rich source of carbon and nitrogen from sea animal excrement, which can increase soil phosphatase activity and thus accelerates the release of phosphorus into tundra ecosystems.

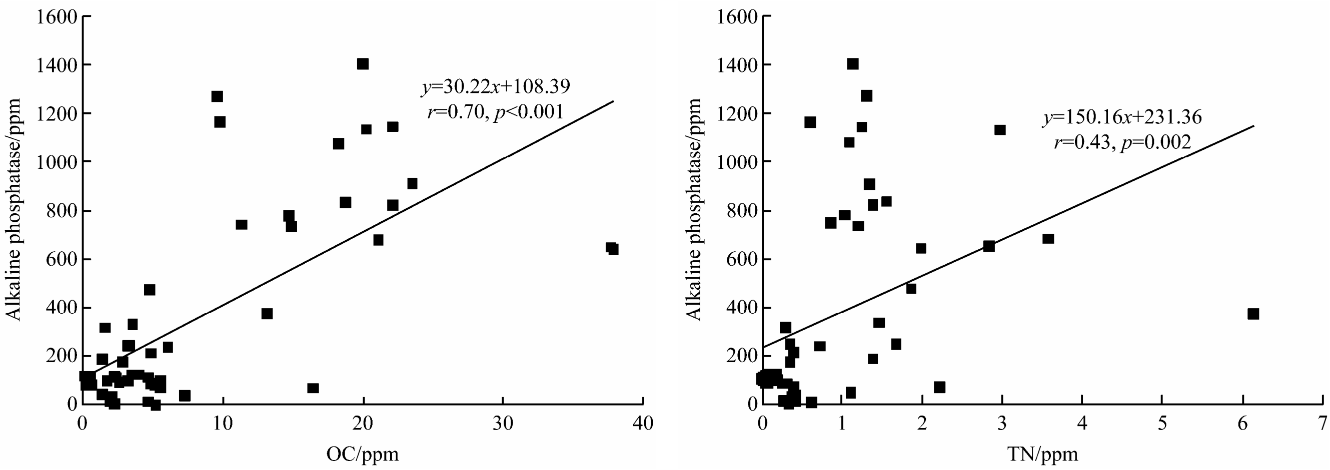

Figure 4 Correlations between APA and OC, APA and TN in all the ornithogenic profiles.

2.3 Relationships between APA and IP, APA and OP, APA and TP

Penguin guano and ornithogenic soils are important environmental matrices rich in phosphorus in the Antarctic tundra environment, and they have a particularly high OP and IP concentration which ranges from 2.0% to 6.2% for penguin guano and from 2.0% to 6.6% in the soils[22]. According to the differences at the sampling sites and soil physical and chemical properties (Table 1), all the ornithogenic profiles can been categorized into the following two groups after the correlation analyses were performed: The first group is a cluster containing the ornithogenic soil profiles DAP, DM1, ZOL, DG2 and DQ; while the second group contains the ornithogenic lake sediment profiles L1, ZH3 and ND.

Alkaline phosphatase may hydrolyze organic phosphorus into inorganic phosphorus. Therefore, theoretically, alkaline phosphatase activity should be positively correlated with inorganic phosphorus concentration. That is, the higher the enzyme activity, the higher inorganic phosphorus concentration[23]. As shown Figure 5, APA showed a significant positive correlation with IP in the ornithogenic soil profiles (r =0.40, p =0.037) while this correlation was not significant in the ornithogenic sediments.

Generally, organic phosphorus increased phosphatase

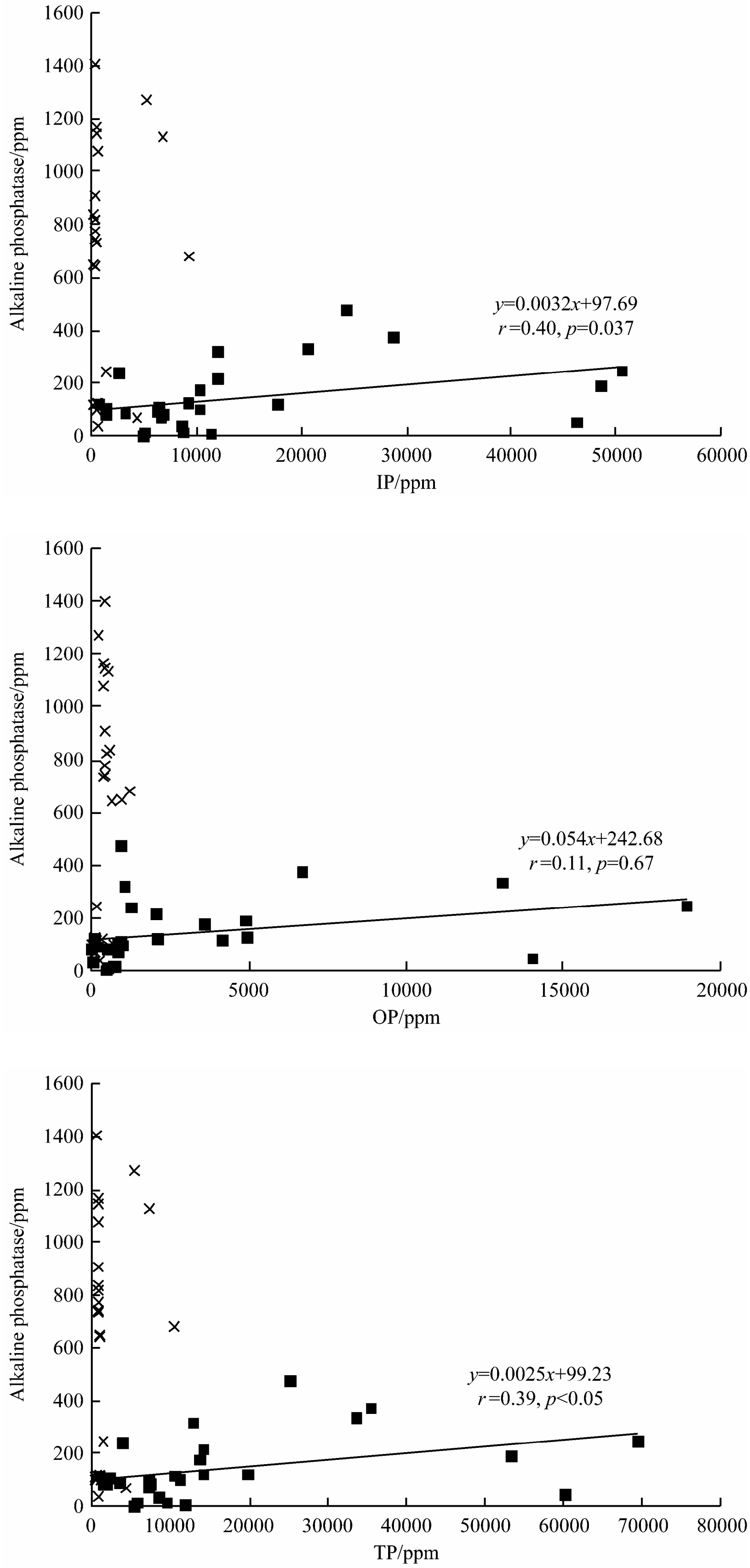

Figure 5 Relationships between APA and IP, APA and OP, APA and TP in all the ornithogenic profiles (■Sampling sites for ornithogenic soils; ×Sampling sites for the ornithogenic Lake sediments).

activity through the induction effects, and soil organic phosphorus content had a close relationship with phosphatase activity[30]. However, in this study APA did not significantly correlate with organic phosphorus in the ornithogenic soils (Figure 5, r =0.11, p =0.67). Similarly, they showed no significant correlation in the ornithogenic sediments. Xue et al.[24] found that APA was positively correlated with OP content if IP (which was produced through the decomposition of OP via alkaline phosphatase) was utilized by the organisms in the sediments. However, APA was negatively correlated with OP content if IP was produced through OP via alkaline phosphatase and released into the overlying water in the sediments. Therefore, no significant correlation between APA and OP was due to the combined effects of the two aspects above especially for the ornithogenic sediments.

Similarly, a significant positive correlation was also found between APA and TP (Figure 5, r = 0.39, p <0.05) for the ornithogenic soils. However, such a correlation was not found for ornithogenic sediments. In polar tundra, the size of the phosphorus fractions in the soils or sediments is an important indicator of fertility, which can affect microorganism populations, some of which produce the phosphatase enzyme, thus affecting APA in the soils or sediments[24]. To sum up, APA is positively correlated with the size of the phosphorus fractions for the polar ornithogenic soils. However, no correlation was obtained for ornithogenic sediments.

2.4 Relationships between APA and the levels of Cu and Zn

As illustrated in Figure 6, APA showed a significant negative correlation with Cu concentrations (r = 0.38, p < 0.05) and Zn (r = 0.28, p = 0.05).

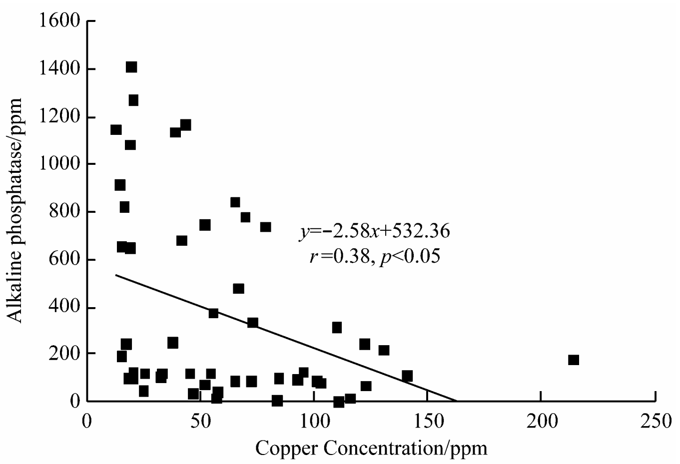

Figure 6 Correlations between APA and Cu, Zn concentrations in all the profiles.

Many studies have shown that heavy metals have an important effect on soil microorganism populations and phosphatase activity. Inhibitory effects of heavy metals on soil enzymes is mainly through their reaction with the mixture of the enzyme and substrate and the combination of heavy metals with the active sections of the enzyme protein. Almost all the heavy metals can inhibit the phosphatase activity in the soils, however, the inhibitory effect depends on the soil type and heavy metal concentrations[15-17]. Juma and Tabatabai[18] added twenty kinds of heavy metals to their soils, and they all could inhibit the activities of acidic and alkaline phosphatase. Likewise, Zhang et al.[14] reported that long-term heavy metal contamination could inhibit the dehydrogenase activity in the soils. In addition, Cu and Zn were significantly enriched in penguin guano and seal excrements, and their levels in ornithogenic soils were significantly affected by inputs from excrement from penguins and seals[20]. Phosphatase is one kind of extracellular enzyme and it exists not only in living microbial cells, but also can be secreted outside of the cells and accumulates in the soils[33]. Some authors have also shown that Cu and Zn has an inhibitory or destructive effect on phosphatase activity[13-14]. Heavy metals also affect the size of soil microbial population[14]. Therefore, high concentrations of Cu and Zn can affect the number of the bacteria cycling phosphorus and thus reduce the secretion of phosphatase. In addition, in our study Cu had a stronger inhibitory effect on APA than Zn in the profiles, which is in accordance with the literature[14].

3 Conclusions

(1) Alkaline phosphatase activity showed a similar distribution pattern with depth in all the ornithogenic soil profiles. The maximum occurred in the surface layers and

then APA decreased with depth.

(2) Alkaline phosphatase activity was significantly positively correlated with the concentrations of OC, TN and phosphorus fractions in the soil, suggesting that C, N, P and organic matter contents are the main factors affecting APA in polar ornithogenic soils/sediments, and soil phosphatase activity can be used as an indicator of soil fertility.

(3) Alkaline phosphatase activity and soil Cu and Zn showed a significant negative correlation, suggesting that heavy metals had inhibitory effects on enzyme activity in our soils.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Grant no. 41076124), the Knowledge Innovation Project of Chinese Academy of Sciences (Grant no. KZCX2-YW-QN510), the Fundamental Research Funds for the Central Universities (Grant no. WK2060190007), and the Open Research Foundation of SOA Key Laboratory for Polar Sciences (Grant no. KP2008004). We would like to thank Prof. Youqing Liang for experimental guidance and Dr. Xiaodong Liu for collecting the Arctic samples. We sincerely acknowledge the members of the 22nd Chinese National Antarctic Research Expedition for assistance with sample collection.

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