Front. Earth Sci. China 2009, 3(2): 226–230
DOI 10.1007/s11707-009-0003-y

RESEARCH ARTICLE

A laboratorial study on influence of alkaline and oxidative environment on preservation of *Pinus tabulaeformis* pollen

Fang TIAN[1,2], Xianyong CAO[1,2], Qinghại XU (✉)[1,2,3], Yuecong LI[1,2]

1 College of Resources and Environmental Sciences, Hebei Normal University, Shijiazhuang 050016, China
2 Hebei Key Laboratory of Environmental Change and Ecological Construction, Shijiazhuang 050016, China
3 Key Laboratory of Western China's Environmental System (Ministry of Education), Lanzhou University, Lanzhou 730000, China

Abstract Different sedimentary settings can influence preservation of pollens, which would lead to misinterpretation of fossil pollen spectrum. This study investigates the influence on the preservation of *Pinus tabulaeformis* pollen by simulating alkaline and oxidative environment in the laboratory. There was no obvious change in the content of *Pinus tabulaeformis* pollen while comparing the original with the ones that were immersed with 10% NaOH liquor for ten days, or boiled for five hours, and or boiled with 20%–30% NaOH for one hour, respectively. However, the pollen fossils were obviously corroded and eroded after being boiled with 40% NaOH for one hour and were seriously corroded after five hours. The result indicates that *Pinus tabulaeformis* pollen is quite durable in alkaline environment and heating condition within a shorter period of time, although alkaline environment has a disadvantage for its preservation. We also tested the influence of oxidation on *Pinus tabulaeformis* pollen preservation with $KMnO_4$ as oxidant. The result presents that the number of remaining *Pinus tabulaeformis* pollen grains decreased quickly after being dipped in $KMnO_4$ along with extending the reaction time and reinforcing oxidant. The rate of remnant pollen grains was less than 1% after being dipped with 2% $KMnO_4$ for one hour. It is suggested that oxidative environment has stronger influence on *Pinus tabulaeformis* pollen preservation than alkaline environment.

Keywords *Pinus tabulaeformis* pollen, pollen fossil corrosion, pollen preservation, alkaline environment, oxidative action

Received May 15, 2008; accepted September 29, 2008

E-mail: xuqinghai@mail.hebtu.edu.cn

1 Introduction

Pollen serves as effective index in reconstructing paleoclimate and paleoenvironment. However, pollen preservation is subject to differences in preservation density in different sediment types even if they were deposited in the same time and same region. One of the major reasons relates to the influence of different deposition (or early diagenesis?) conditions on pollen preservation. Therefore, investigating such influence becomes necessary for better interpreting fossil pollen spectrum and associated environment change.

There are many factors that can influence pollen preservation, such as oxidative action, pH and Eh values, wet-dry cycles, animalcule action, and the content of organic substance in different soil types (Li et al., 2005). Different pollen morphologies are also subject to pollen preservation, such as thickness of exinet, content of sporopollenin, and structure and ornament of exine etc. Zhu (1982) found that fungus and calcium carbonate are disadvantageous to pollen preservation. Havinga (1984) found that bacterium and fungus are able to destroy pollen grains. Xu et al. (2005) considered soil pH influences on pollen preservation. Li et al. (2004) proposed that low pollen content of surface sediment in Daihai lake is related to the high pH value of the lake water.

Oxidation action is usually considered as one of the most serious environmental factors in pollen preservation. The longer the pollen is exposed, the higher the possibility of oxygenation (Paul and Floyd, 1964; Tschudy and Scott, 1969; Elsik, 1971; Wang et al., 1990; Campbell, 1999). Therefore, pollen content is usually low in strong oxidative environment such as loess and cultural sediments (Xu et al., 1998; Pan, 1998; Xia et al., 2001; Zhang et al., 2007).

In China, pollen preservation has been given attention while using it as climate proxy. Many researchers found that pollen content is low and arboreal pollen is rare in the loess sediment in the Loess Plateau. Some scholars considered that the original vegetation was dominated by grassland or forest-grassland on the Loess Plateau (Sun et al., 1995; Li et al., 2003). Some proposed that there were diverse original vegetation types in different topographic positions (Lü et al., 2003; Liu et al., 2004). Others believed that there were large forested areas in the period of paleosoil development (Zhao and Huang, 1999; Tang and He, 2004; Li and Sun, 2005). They suggested that the preservation ability of different pollen is not the same, and the loess environment remains at a disadvantage for pollen preservation, resulting in low pollen content and lower arboreal pollen percentage than non-arboreal in the loess sediment. All these arguments are conjectures due to inadequate research on pollen preservation. The present effort is being made to study the influences of varied deposition (or early diagenesis?) conditions on the preservation of pollen taxa through simulation in a laboratory. Since *Pinus* pollen is usually considered as the pollen taxa with strong preservation ability (Cao et al., 2007) and so does ornament of exine, this study particularly examines the influence of alkaline environment and oxidative action on *Pinus tabulaeformis* pollen preservation.

2 Materials and methods

Pinus tabulaeformis pollen was collected within the campus of Hebei Normal University, and sieved through 200 μm mesh to remove large plant fragments. The sample was then treated with acetolysis (H_2SO_4: acetic anhydride = 1:9) and heated in a water bath for three minutes to remove protoplasm. The mixture of pollen and distilled water was stirred for three minutes, then was extracted 10 μl on a glass slide for microscopic counting.

To get a reliable pollen concentration, we counted all pollen grains for five slides. The pollen concentration of each slide is 410 grains/10 μl, 419 grains/10 μl, 419 grains/10 μl, 442 grains/10 μl, and 446 grains/10 μl, respectively, with the average value of 423 grains/10 μl. Pollen mixture was further stirred for three minutes, and then was distributed into glass tubes of 5 ml volume respectively. There were about 500×423 pollen grains in each tube.

In order to simulate the erosion on *Pinus tabulaeformis* pollen by the alkaline environment, we used NaOH liquor with four different concentrations (10%, 20%, 30%, and 40%) as alkalescence. We chose $KMnO_4$ liquor with different concentrations (2%, 1%, 0.5%, 0.2%, and 0.1%) as oxidant to examine its corrosion in oxidative environment by immersion in the liquor.

The pollen samples were mounted on slides again after being treated in different conditions. At least five slides were made for each condition, and were examined and counted under a light microscope-Olympus at 400× magnification. The remainder pollen rate was calculated using the following formula:

$$\text{remainder pollen rate} = \frac{\text{remnant pollen number}}{423} \times 100\%$$

3 Results

3.1 Influence of alkaline environment on *Pinus tabulaeformis* pollen preservation

After immersion in 10% NaOH liquor for ten days and boiled with 10%, 20% and 30% NaOH for five hours, most pollen is hardly broken. After being boiled with 40% NaOH liquor for one hour, most pollen grains were still not broken, but pollen exine became thin and ornament became faint. After being boiled for five hours, ornament began to break and the pollen outline became serrated (Fig. 1). This shows *Pinus tabulaeformis* pollen concentration didn't change obviously after being boiled in NaOH over a short duration, but changed significantly in strong alkaline environment after being boiled over a longer duration, which is disadvantageous to *Pinus tabulaeformis* pollen preservation.

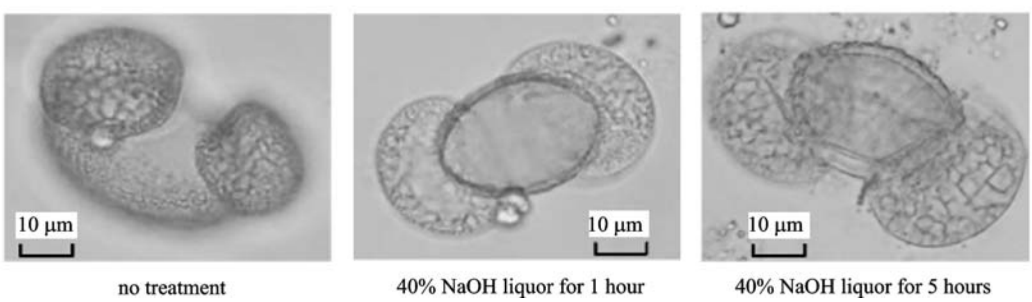

Fig. 1 Change of *Pinus tabulaeformis* pollen grains after boiled in 40% NaOH liquor (Pollen after boiled in lower content NaOH liquor has no obvious change)

3.2 Influence of oxidative environment on *Pinus tabulaeformis* pollen preservation

After immersion with 0.1% $KMnO_4$ liquor, the remainder pollen rate was 65.7% for one hour, decreased to 57.1% for two hours, 52.1% for three hours, 56.6% for five hours (this value of higher than 52.1% may be caused by inevitable error), and 37.5% for six hours. After immersion with 0.1%, 0.2%, 0.5%, 1% and 2% $KMnO_4$ liquor for six hours, the remainder pollen rates were 37.5%, 26.2%, 20.5%, 17.7% and lower than 1% respectively (Fig. 2, 3).

4 Discussion

The present study shows that there was no obvious change in the content of *Pinus tabulaeformis* pollen and most pollen grains were not destroyed if pollen grains were treated with alkaline environment over a short period of time, although pollen exine was corroded after being boiled with 40% NaOH for five hours. This shows *Pinus tabulaeformis* pollen has certain resistance to alkaline environment and heating conditions and can't be destroyed significantly over a short period.

The influence of natural alkaline condition on pollen preservation is a long geological process, while this experiment only lasted for five hours (the longest). Heating can accelerate alkaline action, though it is hard to know how long the heating time in the laboratory is corresponding to geological function time. Short time heating in alkaline condition could not change the pollen, which is in agreement with the viewpoints of previous studies (Wang et al., 1990; Xu et al., 2005). After being boiled with 40% NaOH for five hours, few pollen grains were broken and pollen exine began to corrode, indicating that long-time alkaline environment could be harmful to *Pinus tabulaeformis* pollen preservation.

After immersion with 0.1% $KMnO_4$ for one hour, *Pinus tabulaeformis* pollen grains were corroded significantly. When $KMnO_4$ liquor concentration increased to 2%, most pollen grains were broken after one hour treatment, indicating destruction of *Pinus tabulaeformis* pollen in oxidation environment.

Sunlight and oxygen are the two dominant factors as oxidative function. Surface pollen is affected by oxidative action because of dry climate and strong sunlight in northern China. Therefore, the pollen content that could be identified is generally very low in loose soil of low moisture due to strong oxidative action. In deoxidized lake or marsh environment, the sediments are good carriers of pollen with high pollen content, abundant pollen taxa and explicit ektexine ornament.

Kwaitowski and Mianowska (1957) found that sporopollenin content of *Pinus* was 19.6%, *Betula* 8%, and *Populus* only 5%. Havinga (1964; 1967; 1971; 1984) considered that the higher the content of sporopollenin in the exine, the easier for pollen grain to be preserved in sediments. So, *Pinus tabulaeformis* pollen is easier to preserve than *Betula* and *Populus* pollen. In this experiment, corroded pollen indicates that both alkaline and

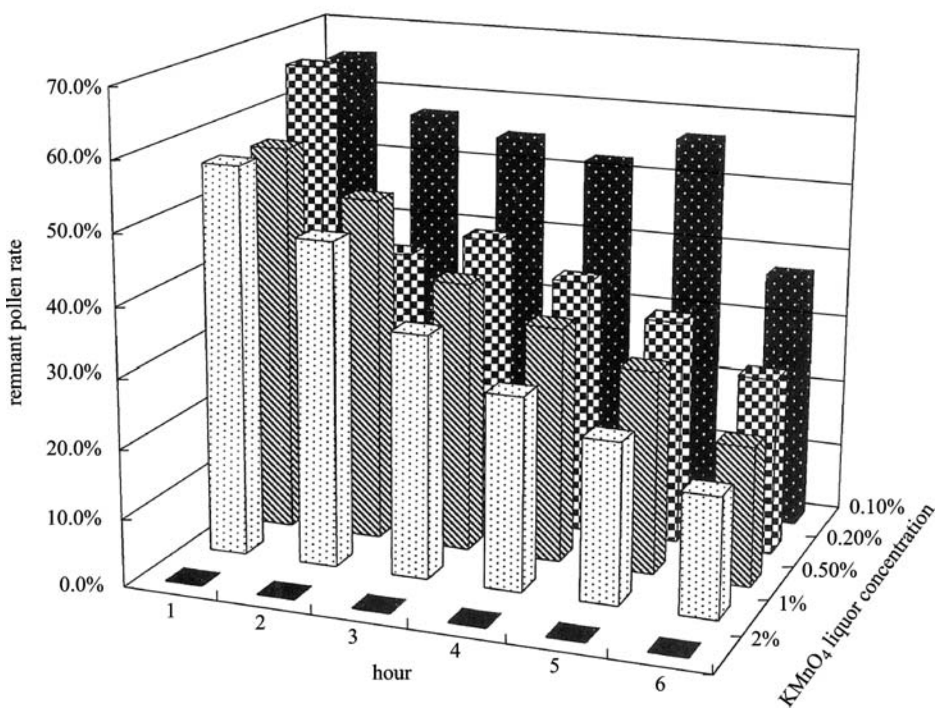

Fig. 2 Remainder pollen rate after treated by $KMnO_4$ liquor

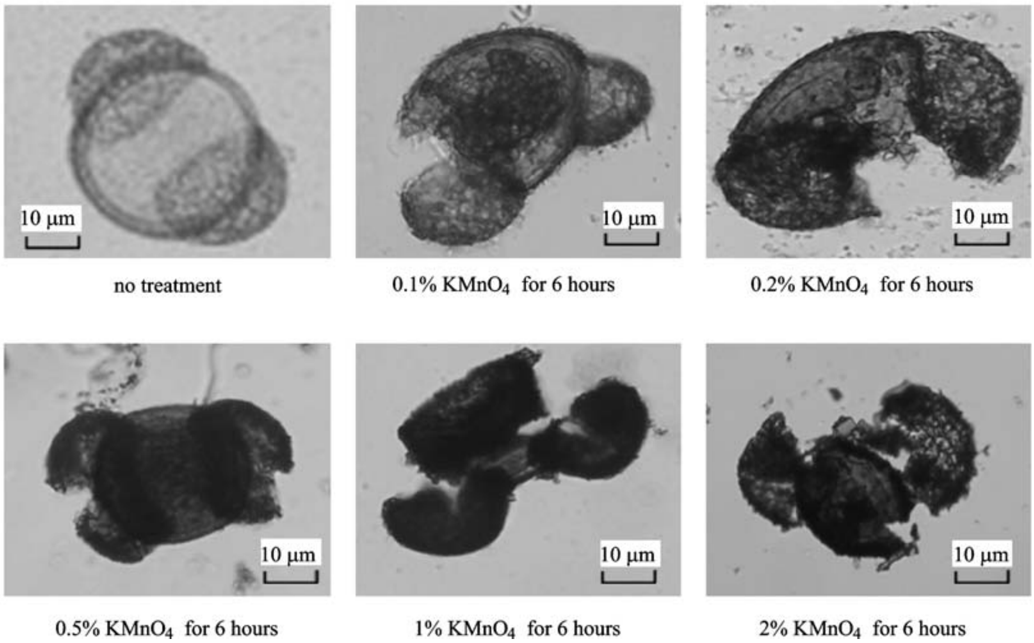

Fig. 3 Comparison for pollen grains immerged in different $KMnO_4$ solution density for six hours

oxidative conditions are disadvantageous to *Pinus tabulaeformis* pollen preservation. But how *Betula*, *Populus* and other pollen types are destroyed in alkaline and oxidative conditions remain to be further questionable.

5 Conclusions

This study proved that *Pinus tabulaeformis* pollen has certain resistance to alkaline and heating condition over a short period, while alkaline environment is keen to corrode the pollen exine and ornament, and is disadvantageous to *Pinus tabulaeformis* pollen preservation.

Oxidative action plays a negative role in devastating *Pinus tabulaeformis* pollen preservation. The remainder pollen rate decreases obviously with reaction time or increase in concentration of $KMnO_4$ liquor. The remainder pollen rate is lower than 1% after being treated with 2% $KMnO_4$ liquor for six hours.

The influence of oxidative action on *Pinus tabulaeformis* pollen preservation is more serious than alkaline environment.

Acknowledgements This research was jointly supported by the Key Program of National Natural Science Foundation of China (Grant No. 40730103), National Natural Science Foundation of China (Grant No. 40571166, 40672107), PhD Foundation of National Education Ministry (Grant No.20050094004), Special Foundation for PhD of Hebei Province (Grant No. 06547006–3), Natural Science Foundation of Hebei Province (D2008000186) and Hebei Foundation for Key Subject Construction of University, Great thank to the two unknown revisers and Dr. Wang Zhanghua at East China normal university for their helpful suggestions on paper structures and English language.

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