和歌山県田辺市及び周辺におけるウメ、ヤマザクラ及び環境における重金属濃度また鉛同位体比の測定

エコ・プジャディ

広島大学大学院生物圏科学研究科

Measurement of heavy metal concentration or lead isotope ratios in tree rings of *Prunus mume* and *Prunus jamasakura* and their environments and around Tanabe City, Wakayama Prefecture, Japan

Eko Pudjadi

Graduate School of Biosphere Sciences, Hiroshima University,
Higashi-Hiroshima 739-8521, Japan

Summary

Chapter 1　Introduction

Tanabe city is number one of the plum (scientifically written by *Prunus mume*) producer in Japan, which produces around 20,000 tons per year, over domestic market. Since 1985, in Tanabe City and its surroundings, the *P. mume* leaves became small and yellow. In the next, the damage of trees spread continue to other trees such as Japanese cedar, *Prunus jamasakura*, etc., and were called as “Growth Difficulty of Trees”. The growth difficulty is confirmed in 10% of the cultivation areas in all over of the prefecture in 1999.

Because of many economical-valued trees has been planted in this region, it is necessary to investigate the possible causes which influenced the growth difficulty. Many research are being done in this city such as is done by Japan Agriculture (JA) and the plum cultivation farmhouse it self. Therefore, today all efforts are done to investigate the causes of growth difficulty of trees, and this study is carried out in order to complete an auxiliary data.

One the other hand, Gobo City where located about 25 km northwest from Tanabe City, had been established Gobo power plant of Kansai Electric Power Company and begun operation in 1984. Smoke of the power plant had been blew-up to region in and around of power plant and may accumulated effluent gradually.

The use of tree rings as temporal monitors of environmental change is possible because trees in temperate regions produce annual growth rings, characterized by a period of rapid growth in spring and followed by a period of slower growth in summer and autumn (Watmough, 1999; Nabais et al.; Marcantonio et al., 1998). Many biomonitor of pollution have been studied in

広島大学総合科学部紀要Ⅳ理系編、第30巻（2004）
＊広島大学審査学位論文
口頭発表日：2004年２月５日、学位取得日：2004年３月23日

Japan, but the study of tree rings for heavy metals monitoring is very little. Indeed, the using of *P. mume* and *P. jamasakura* as biomonitor of pollution may be first time in this region. Thus, this study is carried out in order to describe heavy metals status in tree rings and assess heavy metals in aerosol deposition, soil and precipitation that was sampled around of *P. mume* and *P. jamasakura* stand. In addition, variance component test ($p<0.05$) were performed to assess significance of the difference.

Chapter 2 Study site

The research was carried out at Tanabe City and its surrounding. *P. mume* was sampled at five sites (Kamihaya: 2 sites; Akitsugawa: 2 sites; Kamiakitsu: 1 site) in the northwestern parts of Tanabe City as declined area, and five sites (Nakamisu: 1 site; Shinjo: 2 sites) in the southwestern parts of Tanabe City and Hikigawa-cho (2 sites) as healthy area. *P. jamasakura* was sampled at 2 sites in the northeastern parts of Tanabe City, i.e.

Akitsugawa and Mizukami as declined area, and 2 sites in the eastern parts of Tanabe City, i.e. Fukusada and Housaka Touge as healthy area. The declined areas are outskirts of Tanabe City and located in range 25-30 km from Gobo City. The other areas are far from central of Tanabe City and located keep 40-50 km from Gobo City.

Chapter 3 Method and Experiment

Tree rings sampling: A total of eight disks of damaged 15 and 25 year-old tree rings of *P. mume* were sampled at Kamihaya (2 disks), Akisugawa (2 disks) and Kamiakitsu (1 disk). Also, a total of eight disks of healthy trees were sampled at Nakamisu (1 disk), Shinjo (2 disks) and Hikigawa-cho (2 disks). Disk samples were sampled at 30-50 cm in height of tree and put in vinyl plastic, and packed by carton box.

A total of 36 tree ring samples of 40 years-old of *P. jamasakura* was sampled at Akitsugawa (3 trees) and Mizukami (3 trees). A total of 36 tree ring samples of 50 years-old trees were sampled at Fukusada (3 trees) and Housaka Touge (3 trees). Tree ring samples were taken at six points of each tree with 1-1.5 m in height and packed with aluminum foil, then transport to Hiroshima University.

Aerosol, rain water and soil sampling: The amount of heavy metals in aerosol adhered to vinyl chloride sheets were collected at each *P. mume* stand in both areas (declined area: 1 site, healthy area: 1 site) during one year (1998-1999). Rain samples were collected monthly using a teflon bottle equipped with a teflon funnel in both *P. mume* and *P. jamasakura* stand. Soil samples were taken at areas where tree rings were sampled in *P. jamasakura*, examine stainless steel soil core sampler in 5 cm length and diameter at 5 cm in depth from ground surface on January 9-10, 2002. Samples were sealed by plastic tape to keep samples from contamination and transported to laboratory.

Analysis: The *P. mume* disk samples were taken from plastic bag dried naturally. This process is to make annual growth rings clearer and to make the slice easier. Next, the samples were sliced by a tip saw, in the from of disk samples with a thickness 10 cm. The both surfaces were sliced. If the diameter of disks were too large, they were divided into a few segments in advance. The disks were sliced again to a thickness of 2 to 3 mm, in order to determine annual ring width. Finally, tree ring chips were put on a lamp to make boundaries clearer and annual rings width was measured by microscope.

The *P. mume* tree ring samples were taken from tree ring box and dried naturally. Next, tree ring samples were put at paper and glued by vinyl tape. Annual ring width was measured by loop.

We used nitric acid for all sample preparation. All apparatus and container (consist of Teflon beakers and flasks) were washed and soak by nitric acid in several variety duration and rinsed with deionized water. Pure water was supplied from Milli-Q water system (Millipore Corp., USA) for dilution.

According annual growth, we took for 5 years integrated sample, and about 0.3~0.4 g tree ring sample are sliced and dried at 80℃ for 48 hours to get dry weight. Samples were dissolved in 10 ml HNO_3 and ashed at hot plate 180℃ by heating in covered beaker for 6-8 hours and subsequently allowed to evaporate until drying up. The residue was dissolved in 10 ml HNO_3 and added Mill-Q water in 50 ml volumetric flask. This solution is ready for analysis by ICP-MS.

Aerosol that adhered to vinyl sheet was cut in size about 2.5 cm x 2.5 cm by ceramic knife. The area of the vinyl sample is measured precisely and put in Teflon beaker. The sample is added 20 ml nitric and allowed to melt in covered beaker by heating on the hot plate at 100℃ during 12 hours. The melted sample was dissolved in 5 ml nitric acid and put in volumetric flask, and then added the inside standard element Y, Bi, In, Pt become 5 ppb by Milli-Q water.

Rain water sample was measured pH and filtered with 0.45 μm PTFE membrane filter. Samples were acidified to about pH 5 for determination of heavy metals.

Soil sample treatment is examined to get soluble heavy metal that is ready for chemical uptake. 5 g soil sample and 167 ml water in pH 5.8-6.3 was shake for 6 hours at 20℃. The mixture was filtered by glass fiber filter 0.45 μm. Analysis was examined by ICP-MS.

Chapter 4　Tree ring width of *P. mume* and its heavy metals concentration

Annual ring width of declined *P. mume* decreased to ca. 1 mm since 1990s. In the last 1990s the decreasing of annual ring width occurred significantly in tree rings of the declined 25 years-old and 15 years-old *P. mume*, but not in healthy trees ($p<0.01$). Analysis of cross section area in both trees was also showed significantly difference between declined and healthy trees in the last 1990s ($p<0.01$).

Heavy metals concentration has an increasing trend on five years average in declined tees, but not in healthy trees and so, the difference in the concentration of most heavy metals was significant in the last five years in 1990s. Most of heavy metals concentration in declined trees showed increasing around 1.5~8 times if comparing with healthy trees. The highest difference was V concentration that reached 9.3 times higher in declined trees. V concentration in declined trees has range 12.7~25.3 ng/g, Pb was 4.8~25.5 μg/g and Cu was 2.3~8.3 μg/g.

Chapter 5 Tree ring width of *P. jamasakura*, heavy metals concentration and its lead isotope ratios

Annual ring width sampled at Akisugawa and Mizukami as declined trees decreased to 1.6 mm in the middle 1980s until 1990s. The difference was significant between declined and healthy trees ($p<0.01$). The decreasing of annual ring width in declined trees was about twice if comparing with healthy trees quantitatively.

Heavy metals concentration on five years average in declined trees has a decreasing trend after 1970s and then increased in 1980s. Heavy metals concentration in declined trees is higher than in healthy trees and the differences are significant ($p<0.01$). The increasing of heavy metals concentration of declined trees was 1.3~2.7 times higher than healthy trees. In period 1983-1987, tree ring of declined trees recorded that Cu concentrations has range 1.5~3.7 μg/g, Pb was 0.6~1.0 μg/g and V was 14.9~33.2 ng/g.

Lead isotope ratios in tree rings of *P. jamasakura* sampled at declined area showed variation by year to year. The hard variation of lead isotope ratios in tree rings of *P. jamasakura* indicated that declined areas are more contamination than healthy areas. Lead isotope ratios in declined trees have wide range. Range of $^{207}Pb/^{206}Pb$ ratio in declined trees was 0.848~0.861 and 2.081~2.109 for $^{208}Pb/^{206}Pb$. Healthy trees have $^{207}Pb/^{206}Pb$ ratio in range 0.849~0.854 and 2.082~2.098 for $^{208}Pb/^{206}Pb$.

Chapter 6 Heavy metals concentration in aerosol, rain water and soil

The amounts of heavy metals adhered to vinyl sheet showed concentration in declined area was higher than in healthy area. Comparison of heavy metals concentration between declined and healthy area suggests that Cd, Cu, Ni, Zn, Pb, V and Mn were 6.0, 8.2, 2.1, 2.4, 2.5, 3.8 and 7.3 times in declined area respectively. In addition, the difference of this heavy metals deposition was significant (Cd, Cu, V, Zn, $p<0.01$; Ni, Pb and Mn, $p<0.05$).

Rainwater was sampled monthly during July 2001 until June 2002. Annual precipitation volume during 2001-2002 was recorded 1909 mm (data from JA Kinan, Tanabe City). Annual wet deposition of heavy metals by rainwater showed that declined area has still higher concentration than healthy area. We used single-factor of variance (ANOVA) to test individual heavy metals and showed that most heavy metals were significant variance between declined and healthy area (Cu and Pb, $p<0.05$; and Zn, $p<0.01$). Annual heavy metals concentration in rainwater recorded maximum concentration as 0.51 μg/L for V at declined area, and it means occurred increasing 1.7 times if compare to healthy area ($p<0.05$).

Soil that was sampled in declined area of *P. jamasakura* showed high heavy metals concentration for all heavy metals. Linearity coefficients showed strong correlation between soil and tree rings of *P. jamasakura*. Correlation coefficients (r^2) are 0.71 for Cd, 0.98 for Cu, 0.80 for Ni, 0.93 for Zn, 0.91 for Pb and 0.96 for V. It means that concentration changes of heavy metals in tree rings associated to increased heavy metals in soil.

Chapter 7 Total discussion

Both of *P. mume* and *P. jamasakura* in declined area indicated decreasing in annual ring width since the middle 1980s. Prior to 1980s annual ring width in declined and healthy area was not so difference, around 4~5 mm in *P. mume* and 2.6~3 mm in *P. jamasakura*. Early 1990s annual ring width decreased to 2 mm and enters to 1997 annual ring width in *P. mume* became 1mm. On the other hand, most heavy metals concentration in declined trees have an increasing trend since the middle 1980s and the increasing was significantly high in last five year of 1990s. The remarkable decreasing trend of the annual ring width and increasing tendency of the heavy metals concentration in tree ring were indicated as the characteristics of the declined process for *P. mume* and *P. jamasakura* under such heavy metal conditions in air and soil.

Heavy metals of anthropogenic origin are commonly associated with particles less than 1 μm, which have long atmospheric residence times and small deposition velocities. These particles are most often derived from high temperature combustion sources such as coal-fired power plants, smelters and automobiles (Roy and Negrel, 2001). It is possible to compare heavy metals deposition between declined and healthy area. The amount of heavy metals adhered to vinyl sheet found that declined area occurred heavy metals deposition 2.1~8.2 times higher than in healthy one. In addition, aerosol-deposition gauge was also set up in both areas and made the result as a good correlation to measurement by vinyl sheet (r^2=0.98~0.99). Thus, it supposes that atmospheric heavy metals are likely to contribute the decreasing of annual ring width in *P. mume* and *P. jamasakura*.

The wide range of lead isotope ratios in declined area may link to spread of Pb signature. Variation of Pb isotopes may be caused by mixture of contaminant and natural Pb in soil and ground water. On the contrary, within healthy areas, the natural Pb isotopes signature of groundwater and soil to be similar and invariable through the time.

Heavy metals concentration in soil and rainwater as the recently condition show that concentration is still high in declined area. It seems that heavy metals condition in environment performed characteristics in tree ring of *P. mume* and *P. jamasakura* in declined area.