DISTRIBUTION OF MANGANESE AND COPPER IN GAPs VINEYARD AND ITS POTENTIAL ENVIRONMENTAL RISK: A CASE STUDY AT VINEYARD IN PAK CHONG DISTRICT, NAKHONRATCHASIMA PROVINCE

SUNTHARARAT SAKTONG

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ABSTRACT

A study on manganese (Mn) and copper (Cu) distribution in the environment from the application of agrochemical was performed on a Good Agricultural Practices (GAPs) vineyard located in Pak Chong District, Nakhonratchasima Province, Thailand. The investigation was conducted on a contamination of these pollutants on environmental compartments focusing on surface soil, sediment and grape fruit grown in the study area. Potential environmental risks were evaluated using hazard quotient equation (HQ), and bioaccumulation in grape was also investigated.

In the vineyard soil, Mn content ranged from 1,029 to 3,578 mg/kg dw whereas Cu content ranged from 28 to 83 mg/kg dw and was elevated above background levels and soil quality standards (metals in soil had no correlation with metals in grape fruit). In addition, low bioaccumulation of Mn and Cu in grape fruit resulted from the bioavailable form of Mn and Cu. The concentrations in grape ranged from 0.79 to 3.82 mg/kg ww and 0.39 to 1.82 mg/kg ww, respectively. For sediment in the drainage system of the vineyard, Mn and Cu contents ranged from 526 to 4,490 mg/kg dw and 21 to 36 mg/kg dw, respectively, and was elevated above background levels and sediment quality standards. The pollutants could be leached or washed out from surface soil and transported to the aquatic track. Distribution of pollutants was investigated in the sediment of the receiving reservoir of the vineyard as well as the outlet that flows directly into the public stream about 1 km from the study area. The results showed that Mn and Cu were distributed not far from the contaminated site within a distance of 500 m from the study area. It can be concluded that sediment of vineyard had a high capability for retention of metals in sediment compartments, therefore these two heavy metals might be preferably accumulated in sediment in the aquatic track in the study area, than distributed far from the vineyard.

It can be concluded that the accumulation of Mn and Cu in soil and sediment compartments were elevated greater than the standards (HQ>1) and indicated the ecological risks at the site from viticultural activities. The pollutants exist at a high concentration as viticultural activities have operated for long period of time. However, Mn and Cu exist in the environment largely in the non bioavailable form, which is influenced by site characteristics of soil and sediment. It had no toxic effects on living organisms.

KEY WORDS: MANGANESE/ COPPER/ GAPs IN VINEYARD/ ACCUMULATION/ HQ

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การแพร่กระจายของแมงกานีสและทองแดงในไร่องุ่นที่มีระบบการจัดการเกษตรที่ดี กรณีศึกษา: ไร่องุ่นอำเภอปากช่อง จังหวัดนครราชสีมา (DISTRIBUTION OF MANGANESE AND COPPER IN GAPs VINEYARD AND ITS POTENTIAL ENVIRONMENTAL RISK: A CASE STUDY AT VINEYARD IN PAK CHONG DISTRICT, NAKHONRATCHASIMA PROVINCE)

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบการปนเปื้อน และการแพร่กระจายของโลหะหนักในสิ่งแวดล้อมจากการใช้สารเคมีกำจัดศัตรูพืช และวัสดุปรับปรุงดินในพื้นที่เกษตรกรรม กรณีศึกษาไร่องุ่น อำเภอปากช่อง จังหวัดนครราชสีมา ซึ่งดำเนินกิจกรรมทางการเกษตรด้วยระบบการจัดการเกษตรที่ดี (Good agricultural practices-GAPs) โลหะหนักที่ศึกษาได้แก่ แมงกานีสและทองแดง ซึ่งเป็นองค์ประกอบที่สำคัญในปุ๋ยและสารเคมีกำจัดศัตรูพืชที่ใช้ในไร่องุ่นนี้ โดยเน้นการปนเปื้อนในดิน ตะกอนดิน และผลผลิตทางการเกษตร (ผลองุ่น) ความเสี่ยงต่อสิ่งแวดล้อม (Environmental potential risks) ของโลหะนี้ถูกประเมินโดยใช้สมการ Hazard Quotient (HQ) และมีการตรวจสอบปริมาณโลหะทั้งสองที่สะสมในเนื้อเยื่อของผลองุ่นในพื้นที่ศึกษาด้วย

ในดินของไร่องุ่นที่ทำการศึกษา พบว่ามีปริมาณแมงกานีสและทองแดงอยู่ในช่วง 1,029 ถึง 3,578 มก./กก. และ 28 ถึง 83 มก./กก. ตามลำดับ ซึ่งมีค่าสูงเมื่อเปรียบเทียบกับดินในพื้นที่ศึกษาที่มิได้มีการปนเปื้อนและค่ามาตรฐานโลหะหนักในดิน และเมื่อเปรียบเทียบปริมาณโลหะทั้งสองชนิดในดินและผลองุ่น พบว่าไม่มีความสัมพันธ์กัน โดยปริมาณแมงกานีสและทองแดงที่สะสมในเนื้อเยื่อองุ่นมีค่าต่ำที่ระดับความเข้มข้น 0.79 ถึง 3.82 มก./กก. และ 0.39 ถึง 1.82 มก./กก. ตามลำดับ ทั้งนี้เนื่องจากลักษณะทางกายภาพและเคมีของดินทำให้โลหะอยู่ในรูปที่ไม่ละลายและรากพืชไม่สามารถนำไปใช้ได้ และเมื่อพิจารณาตะกอนดินซึ่งเป็นแหล่งสะสมของสารมลพิษจากการชะละลายของหน้าดิน พบว่าตะกอนดินภายในพื้นที่ศึกษามีปริมาณแมงกานีสและทองแดง 526 ถึง 4,490 มก./กก. และ 21 ถึง 36 มก./กก. ตามลำดับ ซึ่งสูงกว่าตะกอนดินในลำธารสาธารณะและค่ามาตรฐานโลหะหนักในตะกอนดิน เมื่อวิเคราะห์การกระจายตัวของโลหะทั้งสองในตะกอนดินในระยะทาง 1 กิโลเมตรจากไร่องุ่น พบว่าโลหะหนักทั้งสองนั้นมีการกระจายตัวต่ำและสิ้นสุดที่ระยะทางไม่เกิน 500 เมตรจากไร่องุ่น โดยอธิบายได้จากลักษณะทางกายภาพและเคมีของตะกอนดินในพื้นที่ศึกษา ซึ่งมีความสามารถในการยึดจับกับโลหะได้เป็นอย่างดี ทำให้โลหะทั้งสองชนิดตกสะสมในตะกอนดินในไร่องุ่นและกระจายได้ไม่ไกลจากพื้นที่ศึกษา

การศึกษานี้สรุปได้ว่า การสะสมของแมงกานีสและทองแดงในดินและตะกอนดินมีค่าเกินมาตรฐาน (HQ>1) แสดงว่าระบบนิเวศในพื้นที่ศึกษามีความเสี่ยงจากการทำไร่องุ่น ซึ่งปริมาณแมงกานีสและทองแดงมีค่ามากขึ้นเมื่อดำเนินกิจกรรมทางการเกษตรติดต่อกันเป็นระยะเวลานาน อย่างไรก็ตามแมงกานีสและทองแดงในสิ่งแวดล้อมของพื้นที่ศึกษาส่วนใหญ่อยู่ในรูปที่สิ่งมีชีวิตไม่สามารถนำไปใช้ได้ เนื่องจากลักษณะของดินและตะกอนดินในพื้นที่ที่ศึกษา ทำให้แมงกานีสและทองแดงไม่เป็นพิษต่อสิ่งมีชีวิต

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LIST OF ABBREVIATIONS

°C = Degree Centigrade
CCME = Canadian Council of Minister of Environment
CEC = Cation Exchange Capacity
cm = Centimeter
Cu = Copper
dw = Dry weight
FAAS = Flam Atomic Absorption Spectrophotometer
g = Gram
GAPs = Good Agricultural Practices
HQ = Hazard Quotient
hr = Hour
kg = Kilogram
km = Kilometer
m = Meter
mg = Milligram
mg/kg = Milligram per Kilogram
ml = Milliliter
mm = Millimeter
Mn = Manganese
nm = Nanometer
OM = Organic matter
PCD = Pollution Control Department
US EPA = United States Environmental Protection Agency
ww = Wet weight

CHAPTER I
INTRODUCTION

1.1 Statement of the Problem

Heavy metal contaminations in soil are the important problem because their properties are non-biodegradable, non-thermodegradable and such soil purifies slowly which these properties cause heavy metal persistence in soil environment. Although these pollutants are ubiquitous in soil parent materials, significant problem can occur when exceeding concentrations from anthropogenic processes. Agricultural practices are the process that constitutes very important non-point sources of heavy metal. These make significant contributions to their concentrations in agricultural soils in many parts of Thailand, especially in vine growing regions.

Agrochemical products and organic amendments are the main source of these pollutants which distribute to soil in term of an impurity in fertilizers, lime materials, farmyard manures and finally form a part of active component of pesticides. Viticulture is known to be the area of intensive use of agrochemical products. It was a line of thought for study on heavy metal contamination in vineyard soil. The field study was performed on a vineyard of northeast vine growing region located at Pak Chong District, Nakhonratchasima Province. The vineyard has been managed in the manner of Good Agricultural Practices (GAPs) for nineteen year more. In this manner, the production processes should play safe for producers and consumer. It includes the sufficient used of natural resources and establishment of sustainable agriculture systems which may reduce the contamination of hazardous materials in the environment.

The study found that manganese (Mn) and copper (Cu) are the active ingredient in pesticides which have been used for a decade in this vineyard. Mn is from a part of active ingredients of mancozeb whereas Cu is the active ingredient of copper oxychloride ($CuCl_2 \cdot 3Cu(OH)_2$). Those of chemicals are used as fungicide which are low toxic and quickly degradable. But Mn and Cu in their molecules are non-biodegradable and such persist in soil environment for long period of time.

Moreover, Mn and Cu are presented in a large amount in fertilizers and organic amendments. Typical ranges of Mn and Cu in phosphate fertilizers are 40-2,000 mg/kg and 1-300 mg/kg, respectively, in farmyard manure, Mn and Cu are ranged 30-969 mg/kg and 2-172 mg/kg, respectively (Alloway, 1995). In this study, the investigation on heavy metal contamination in vineyard soil was conducted on Mn and Cu based on the uses of agrochemical and GAPs management in the vineyard.

The repeated uses of these chemicals to control cultivated plant disease and soil nutrient improvement have led to long term accumulation of Mn and Cu in soils resulting in contaminations in sediment and crop product consequentially. The processes can affect soil microorganisms, human health, though soil ingestion or through the food web by animal and crop ingestion. Mn and Cu are the essential element in biological function as a micronutrient for plant and animal growth, component in cells and essential for metabolisms. However, they become toxic to all living organisms when the concentrations are extremely high. Furthermore, it is known that serious systemic health problems can develop as a result of intense accumulation of dietary heavy metals in human body. Generally, the level of Mn and Cu in soil are ranged from 500-900 mg/kg (IPCS, 1981) and 2-250 mg/kg (IPCS, 1998), respectively.

In this study, the purpose was to investigate the distribution of Mn and Cu from agrochemical applications in environmental compartments of the vineyard focusing on surface soil, sediment and crop product. The potential environmental risks were evaluated in soil and sediment using Hazard Quotient equation (HQ) (US EPA, 1997) and a bioaccumulation in crop product (grape fruit) was investigated as well. The results can be used as the baseline data in agricultural management of GAPs and such the problem of intensive use of pesticide and fertilizer that might be occurred in the future can be then prevented.

1.2 Research Objectives

1.2.1 General objective

The purpose of this study was to investigate the distribution of Mn and Cu from the applications of agrochemicals in GAPs vineyard located at Pak Chong District, Nakhonratchasima Province.

1.2.2 Specific objectives

1.2.2.1 To determine the contaminated concentrations of Mn and Cu in surface soil as well as in sediment of the vineyard which exhibited as a reservoir of accumulation

1.2.2.2 To investigate the distribution of Mn and Cu in the aquatic track of the study by determining Mn and Cu concentration in sediment at effluent discharge points down to the public stream

1.2.2.3 To investigate the bioaccumulation of Mn and Cu in crop product by determining the concentration of Mn and Cu in grape fruit grown in study area

1.2.2.4 To evaluate the potential environmental risks of Mn and Cu focusing on surface soil and sediment using Hazard Quotient equation (HQ) (US EPA, 1997)

1.3 Research Hypotheses

1.3.1 Mn and Cu can be contaminated and accumulated with elevated concentrations in this vineyard when the site has cultivated over a long period of time

1.3.2 The distribution of Mn and Cu in an aquatic track is not far from the study site due to the sediment characteristics presented in high capable retention of metal in sediment component

1.3.3 Mn and Cu can be accumulated in grapes fruit with low concentrations as site characteristics provided non-bioavailable form of metals

1.4 Scope of the Study

The field study was performed in the vineyard of vine growing region at Pak Cong District, Nakhonratchasima Province as a representative of contaminated area from agrochemical applications. Mn and Cu, the active ingredients of pesticides and fertilizers used in the study area, were selected to investigate the contaminations in environmental media including soil, sediment and grape fruit specific to vineyard operated in the manner of GAPs.

1.4.1 The concentrations of Mn and Cu were determined in surface soil, sediment and grape fruit in term of total metal contents.

1.4.2 Soil and sediment were collected at surface (0-5 cm in dept) once in dry season of December, 2006, as a representative of total contamination in this year.

1.4.3 The area of vineyard was divided into three transect lines and randomly selected the three sampling plots of each transect line to investigate the contaminations. The background sites were collected in the same area of vineyard but upwind away from the applications of pesticides and fertilizers and had the same soil characteristics as well as those of contaminated sites.

1.4.4 Sediments were collected from water drainage systems along vine sampling plot inside vineyard and at the effluent discharge point as well as down stream about 1 km outside vineyard.

1.4.5 Physical and chemical properties of soil and sediment were analyzed at the laboratory of Soil and Science at the Office of Science for Land Development, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

1.4.6 Grape fruits were collected from each vine plot at the same location as had done for soil in order to investigated bioaccumulation and crop ingestion/uptaken. The collection was done once during ripen time of March, 2007.

1.5 Definition of Keywords

1.5.1 Viticulture is the cultivation of grapes fruit, often for use in winemaking. It is a part of the agricultural science (Johnson, 1989).

1.5.2 Metal based agrochemicals are compounds which have metal or heavy metal in theirs molecule. In this study, there are pesticides and fertilizers used in agriculture to control cultivated plant diseases and improve nutrient supply in soil, for example, Cu is an active component to inhibit the growth of fungi in copper oxychloride.

1.5.3 GAPs or Good Agricultural Practices are the principles or specific methods applying for on-farm production and post-production processes, resulting in safe, high yielding, account economical and high quality products. The production processes save for the producers and consumers, including sufficient used of natural resources. They also establish sustainable agricultural methods and concern the environment in term of reducing the contamination of hazardous materials in agriculture systems (UN FAO, 2007). Recommendations for GAPs process are using of both low persistence and low toxic substances.

1.5.4 Contaminated site is a site which the presence of man-made chemicals or other hazardous substance is above the natural or background level.

1.5.5 Background site is a site which is uncontaminated with anthropogenic substances as a result of natural geologic occurring and should have the same soil characteristic as those of contaminated site.

1.6 Conceptual Framework

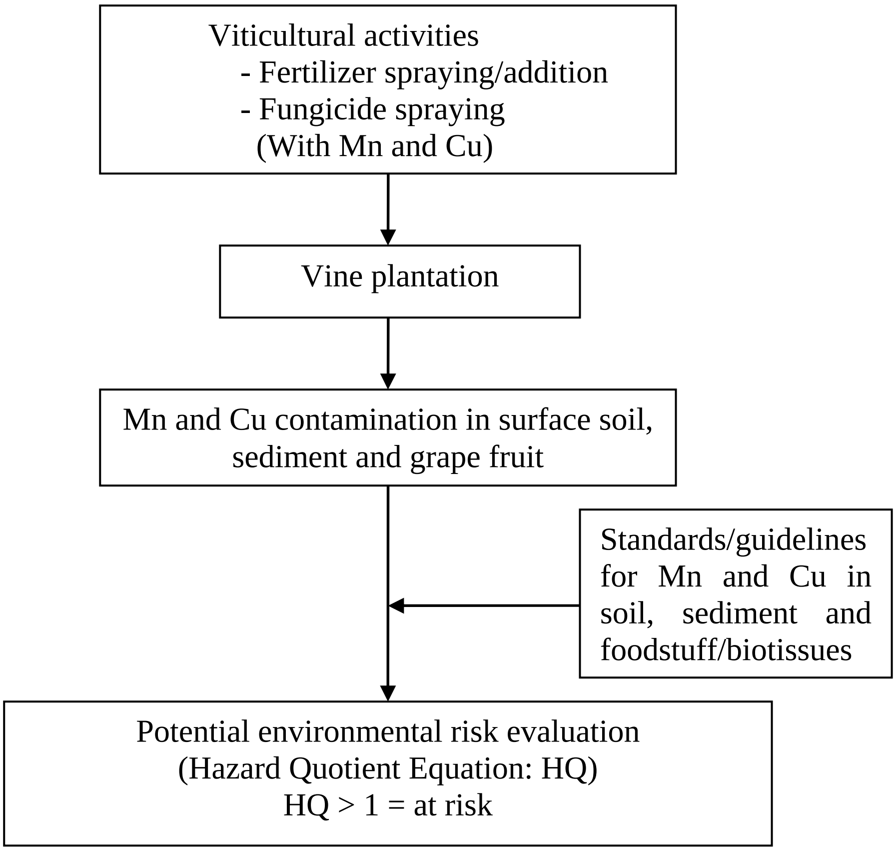


Figure 1.1 Conceptual framework of the study

CHAPTER II
LITERATURE REVIEWS

Heavy metals are metals with a specific gravity more than 5.0, including manganese, copper, zinc, lead, arsenic, cadmium, chromium and mercury (Alloway, 1990). These metals are component in natural environments such as air, water, soil, sediment and bio-tissues as background values and present at high concentrations with the receiving of anthropogenic processes. Agricultural practices are important source of heavy metals. In this study, Mn and Cu were selected to study the characteristics, ecological toxicity and properties in the environment as well as the contamination in soil, sediment and agricultural product.

2.1 The Properties

2.1.1 Manganese

Manganese (Mn) is a naturally occurring element that is found in rock, soil, and water. It is ubiquitous in the environment and comprises about 1% of the Earth's crust. Mn does not occur naturally as a base metal but is a component of more than 100 minerals (IPCS, 1981).

(1) Physical properties

Mn is an element of the VIIb group in periodic table of elements. Mn is a d-block transition element and in period number of 4. Because of the transition element property, Mn can exist in a variety of oxidation states, form many color and paramagnetic compounds. In the elemental state, Mn does not have a special taste or smell. It is a white-gray, pinkish tinge, brittle and reactive metal with high melting and boiling point. Physical properties of Mn are shown in Table 2.1.

Table 2.1 Physical properties of Mn

Properties	**Manganese properties**
Atomic number	25
Atomic mass (g/mol)	54.93805
Density (g/cm^3 at 20°C)	7.43 (solid)
Melting point (°C)	1245.0
Boiling point (°C)	1962.0
Isotope	^{50}Mn, ^{51}Mn, ^{52}Mn, ^{53}Mn, ^{54}Mn, ^{54}Mn, ^{55}Mn, ^{56}Mn, ^{57}Mn
Phase	Solid
Crystal structure	Cubic body centered

Source: Barbalace (2007)

(2) Chemical properties

Mn is a naturally occurring element that is found in rock, soil, water and food, and with combines with other elements such as oxygen, sulfur, carbonate, silicate, phosphate, borate, and chlorine. It can exits in 11 oxidation states ranging from -3 to +7 but the most common oxidation states are Mn^{2+} (e.g., manganese chloride ($MnCl_2$)), Mn^{4+} (e.g. manganese dioxide (MnO_2)), and Mn^{7+} (e.g. potassium permanganate ($KMnO_4$)), depending upon the oxidation-reduction potential (redox potential), pH of the environment and the nature of the anions and other groups present with.

In addition, Mn can be combined with carbon to make organic Mn compounds such as pesticide (maneb and mancozeb), methylcyclopentadienyl manganese tricarbonyl (MMT) which is used as additive to increase the octane level of gasoline and thus improve the antiknock properties of fuel (IPCS, 1981) and mangafodipir (MnDPDP), a contrast agent for magnetic resonance (MR) imaging (ATSDR, 2000).

Mn and its compounds exit in soils as solids and migrate as particulate matters to air or water, or soluble compounds can be leached from soil. In soils, its solubility of Mn is determined by two major variables: pH and redox potential. The exits of Mn in aquatic environment are in two main forms: Mn^{2+} and Mn^{4+}. Movement between forms occurs via redox reactions that may be microbially or abiotic mediated.

The environmental chemistry of Mn is largely governed by pH and redox conditions: Mn^{2+} dominates at low pH and redox potential and with increasing proportion of colloidal manganese oxyhydroxides above pH 5.5 in non-dystrophic waters. In water most Mn compounds are readily soluble, with only carbonate and phosphate having low solubility, and oxides of Mn (e.g. MnO_2 and Mn_3O_4) are poorly soluble in water. Primary chemical factors controlling sedimentary Mn cycling are oxygen content of the overlying water, the penetration of oxygen into the sediments, and benthic organic carbon supply.

2.1.2 Copper

Copper is a metal occurs naturally in rock, soil, water, sediment and at low level in air. The average concentration in the earth' crust is about 50 ppm (50 mg per kg of soil). Copper presents naturally and known to be an essential element in all living organisms.

(1) Physical properties

Copper is the first element of group IB of the Periodic Table that has symbol Cu. It is a ductile and malleable metal which has high thermal conductivity and high electrical conductivity. Cu is a reddish brown metal with high boiling and melting point. It is low corrosion. The physical properties of Cu are shown in Table 2.2.

Table 2.2 Physical properties of Cu

Properties	**Copper properties**
Atomic number	29
Atomic mass (g/mol)	63.546
Density (g/cm^3 at 20°C)	8.96
Melting point (°C)	1,084.62
Boiling point (°C)	2,562
Isotope	^{63}Cu (69.17%), ^{65}Cu (30.83%)
Phase	Solid
Crystal structure	Cubic body centered

Source: IPCS (1998)

(2) Chemical properties

Cu is an element that can be found in four oxidation states: metallic copper (Cu^0), cuprous ion (Cu^+), cupric ion (Cu^{2+}), and trivalent copper ion (Cu^{3+}). Cu also forms as organometallic compounds. It is found in a wide variety of mineral and organic compounds (IPCS, 1998).

Cu can be found naturally in the elemental or metallic form. The metallic form is very stable to dry air at low temperature but undergoes a slow reaction in moist air to produce a hydroxycarbonate or hydroxysulfate that forms a greenish-gray amorphous film over the surface which protects the underlying metal from further attack. The metal is sparingly soluble in water, in salt solution, and in mildly acidic solutions, but can also be dissolved in nitric acid and sulfuric acid as well as in basis solutions of ammonium carbonate and cyanide in the presence of oxygen (Cotton and Wilkinson, 1989).

The common oxidation states of Cu are the cuprous (Cu^+) and cupric (Cu^{2+}) forms. The chemistry of the element, especially in biological systems, is profoundly affected by electronic/oxidation state. The facile change between oxidation states endows the element with oxidation-reduction properties which may be of an essential or deleterious nature in biological systems. Cu^+ is quickly oxidized by any oxidizing reagent, or in a disproportionation reaction in aqueous solution, unless it is stabilized by complex formation. The only Cu^+ compounds that are stable in water are extremely in soluble ones such as cuprous chloride (CuCl). It has been shown that Cu^+ complexes may be formed in seawater by photochemical processes and may persist for several hours. Cu^+ compounds are generally colorless (ATSDR, 2004).

Cu^{2+} is the most importance oxidation state of Cu in natural, especially aqueous environment. Cu^{2+} is the oxidation state of Cu generally encountered in water. Most Cu^{2+}compounds and complexes are blue or green in color. When Cu^{2+} is introduced to the environment, it typically binds to inorganic and organic materials contained within waters, soils, and sediments. In water, Cu^{2+} binds to dissolved organics such as humic or fulvic acids. The Cu^{2+} forms stable complexes with $–NH_2$, -SH, and to a lesser extent, -OH groups of these organic acids. Cu^{2+} will bind to inorganic and organic components in sediments and soils with varying affinities. For examples, Cu^{2+} binds strongly to hydrous manganese and iron oxide in clays and to humic acid in organic matter, much less strongly to aluminosilicates in sand. As in water, the binding affinities of Cu^{2+} with inorganic and organic matter in sediments and soils is depended on pH, the oxidation-reduction potential in the environment, the presence of competing metal ions and inorganic anions such as iron, aluminum, and manganese (Grant et al., 1990).

The trivalent form of Cu is found in only a few compounds and is a strongly oxidizing agent (Cotton and Wilkinson, 1989).

2.2 Environmental Fates

Metals exchange between an ecosystem and biosphere controls by many chemical and bio-chemical processes. Organisms exchange metals with non-living environment. Some metals are taken up from and released to the atmosphere and some are exchange with water, sediment and soil. Plants can adsorb heavy metal in soluble or available forms. The transport of metals in the environment exhibits in Figure 2.1. The mobility and availability of these elements are controlled by chemical and biochemical processes, which are shown in Figure 2.2. The processes are precipitation-dissolution, adsorption-desorption, complexation-dissociation and oxidation-reduction (He et al., 2005).

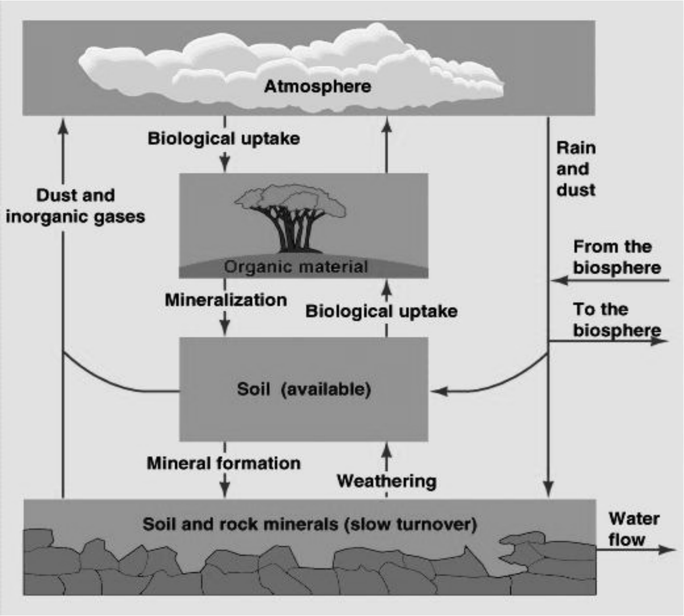


Figure 2.1 The transport of metals in the environment
(VU University Amsterdam, 2003)

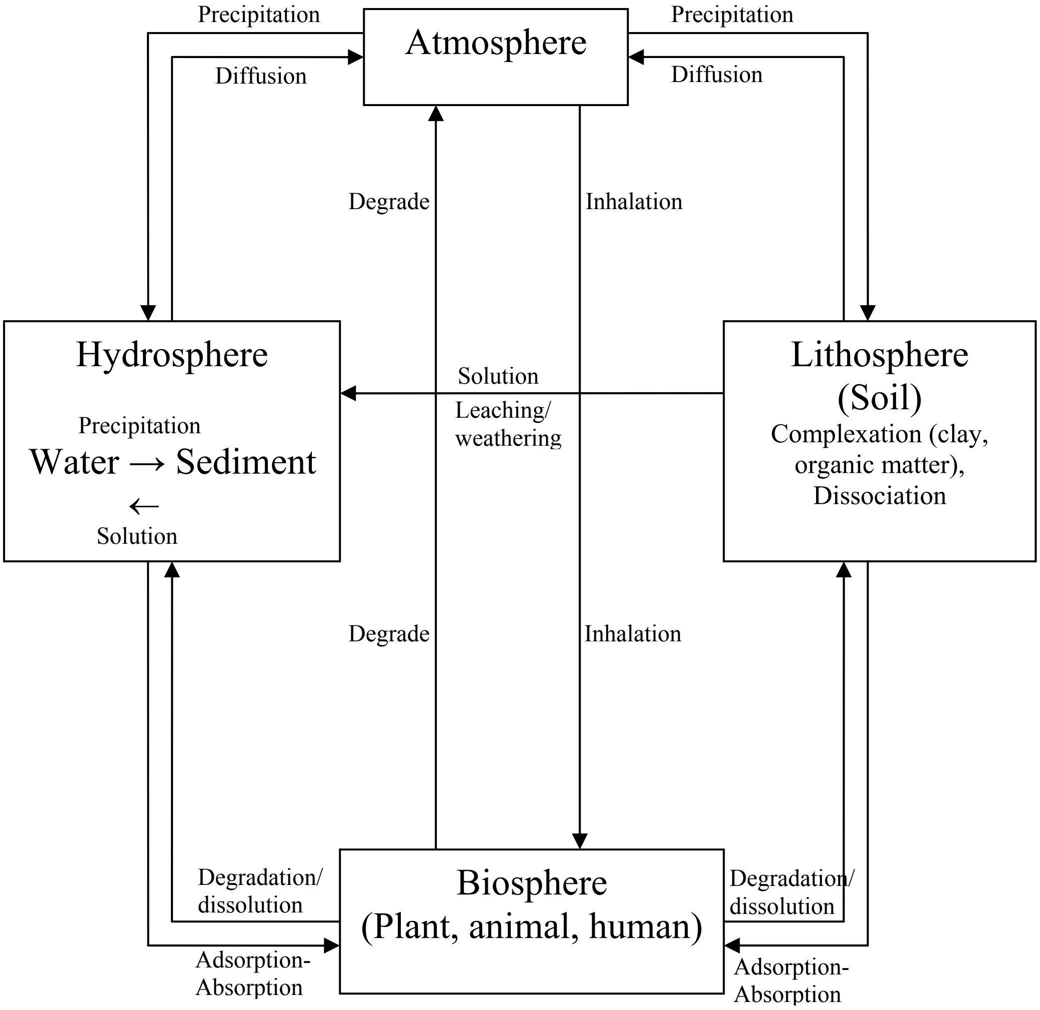


Figure 2.2 The chemical processes for metal mobility
(Adapted from He et al., 2005)

2.2.1 Environmental fates of manganese

(1) Manganese in air

Elemental Mn and inorganic Mn compounds have negligible vapor pressures but can exist in atmosphere as suspended particulate matters, aerosols, and fumes (US EPA, 1984). In troposphere, Mn is likely in form of oxide, sulfate, nitrate, and mineral complexes related to its natural origin in soil or rock (Stokes et al., 1988).

Soil particulate matters containing Mn can be transported into air by weathering and leaching. The fate and transport of Mn particles in air depend on size, density, wind speed, and wind direction. Mn-containing particles are mainly removed from the atmosphere to lithosphere and hydrosphere by gravitational settling or wet and dry deposition, depending on particle size and density. An estimated 80% of Mn in suspended particulate matters is associated with particles with a mass median equivalent diameter of < 5 μm, and 50% of this Mn is estimated to be associated with particles that are < 2 μm.

(2) Manganese in water

Mn can exit in aquatic environment (by atmospheric deposition and soil leaching) in two main forms; Mn^{2+} and Mn^{4+}. Transition between forms occurs via oxidation-reduction potential that may be abiotic or microbially mediated (Heal, 2001). The environmental chemistry of Mn is largely governed by pH and redox conditions. Mn^{2+} dominates at lower pH and redox potential, with an increasing proportion of colloidal Mn oxyhydroxides above pH 5.5 in non-dystrophic waters (LaZerte and Burling, 1990). A complex series of oxidation/precipitation and adsorption reactions occurs when Mn^{2+} is present in aerobic environments, which eventually renders the Mn biologically unavailable as insoluble Mn dioxide (MnO_2). However, the kinetics of Mn^{2+} oxidation is slow in waters with pH below 8.5 (Zaw and Chiswell, 1999). The time required for the oxidation and precipitation of Mn ranges from days in natural waters to years in synthetic waters (Stokes et al., 1988), however, oxidation rates of Mn increase with increasing pH or the presence catalytic surfaces such as MnO_2 (Huntsman and Sunda, 1980). The sequence of reactions involving the oxidation of Mn^{2+} and subsequence precipitation as MnO_2 includes simultaneous occurrence of several Mn forms (such as dissolved Mn^{2+}, hydrous oxides of Mn^{3+}, Mn^{2+} adsorbed to particulates and Mn^{2+}-ligand complexes), with individual concentrations dependent on factors that include pH, inorganic carbon, organic carbon, sulfate, chloride, time and temperature (Stokes et al., 1988). Jaudon et al. (1989) present that Mn^{4+}can be reduced both chemically and bacterially to Mn^{2+} oxidation state in groundwater with low oxygen levels.

There are little evidences for organic Mn associations in natural waters, with Mn only weekly bound to dissolve organic carbon (L'Her Roux et al., 1998). Hence, organic complexation does not play a major role in controlling Mn speciation in natural waters. Field studies have confirmed that organically bound Mn is minor, even with high natural dissolved organic carbon levels (Laxen et al., 1984). The Mn^{2+}

ion is more soluble than Mn^{4+}. Therefore, Mn will tend to become more available with decreasing pH and redox potential (Heal, 2001). The presence of chlorides, sulfates and nitrates can increase Mn solubility and thus increase aqueous mobility and uptake by plants (Reimer, 1999).

Surface freshwater data suggests that higher Mn concentrations occur during periods of higher stream flow, such as spring runoff, and lower concentrations tend to occur downstream of lakes that act as settling areas for sediment (Reimer, 1999).

Mn is often transported in rivers adsorbed to suspended sediments. The tendency of soluble Mn compounds to adsorb to sediments can be highly variable, depending on CEC, the organic composition of the sediments (Hemstock and Low, 1953; Schnitzer, 1969; McBride, 1979; Curtin et al., 1980; Baes and Sharp, 1983; Kabata-Pendias and Pendias, 1984). The particulate and dissolved phases for rivers and streams can be decoupled with weathering processes, leading to suspended sediment and influxes of Mn^{2+} species leaching from anoxic soil and groundwater (Laxen et al., 1984). The speciation in any particular river or stream will depend on the hydrogeological conditions of the catchments at time of sampling. Suspended sediment, with a Mn content dependent upon the catchments geology, will be mixed with Mn^{2+} species in varying proportions. Primary chemical factors controlling sedimentary Mn cycling are the oxygen content of the overlying water, penetration of oxygen into the sediments, and benthic organic carbon supply (Lynn and Bonatti, 1965; Balzer, 1982; Sundby et al., 1986; Hunt and Kelly, 1988). Mn exchange between water and sediment is an interdependent process. A cycle between sediment and water is maintained, since dissolved Mn^{2+} is particle-reactive (Hunt, 1983). Once incorporates into sediments, solid phase manganese oxide (MnO_2) undergo reduction to soluble Mn^{2+} during anaerobic decomposition of organic matter (Pohl et al., 1998). Release from sediment to water by diffusion processes as a result of a steep Mn^{2+} concentration gradient across the sediment pore water and bottom water interface (Balzer, 1982; Kremling, 1983; Jung et al., 1996). Recycling at a redox boundary is involved in the formation of enriched Mn horizons. Mn precipitating on the oxic side of a redox boundary consists of a Mn^{4+}oxide. Removal of Mn^{2+} by diffusion in the pore water is a slow process, and so supersaturation and precipitation of carbonate are likely to occur, transforming labilized oxide to stable carbonate. Under intermittently anoxic conditions, fixation of an enriched horizon may occur by precipitation of MnO_2 from the water column during oxic periods, burial in sediment, and transformation to carbonate (Schaanning et al., 1988). The fate and transport of Mn in water is shown in Figure 2.3.

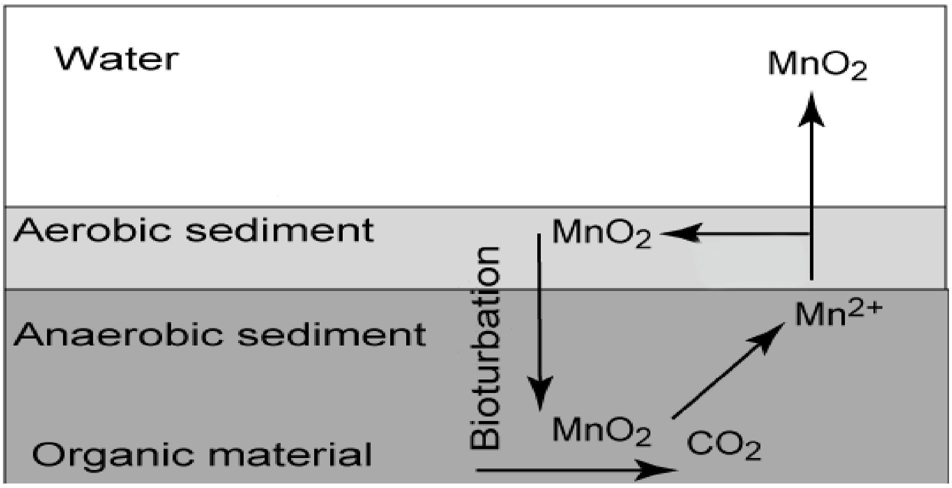


Figure 2.3 Fate and transport of manganese in water and sediment (Adapted from Thamdrup et al., 1994)

(3) Manganese in soil

In soil, Mn solubility is determined by two major variables; pH and redox potential. Water soluble Mn in soils is directly proportional to pH, with oxidation state being another major determinant of Mn solubility. The lower oxidation state, Mn^{2+}, predominates in reducing conditions, resulting in higher concentration of dissolved Mn in flooded soils or other reducing situations (Stokes et al., 1988). This is normally reflected in higher Mn bioavailability in flooded soils. In some situations, however, there is competition by iron and plant absorption of Mn is decreased or unaffected by flooding (IPCS, 1981). The oxidizing power of higher oxides increases with acidity and thus reduction by organic matter is more likely at low pH values. If the oxygen tension is low, biological reduction can take place at any pH value.

The oxidation state of Mn in soils can be altered by microbial activity (Geering et al., 1969; Francis, 1985). Geering et al. (1969) presented that Mn^{2+} in suspensions of silt or clay loams was oxidized by microorganisms, leading to precipitation of Mn minerals. Bacterial oxidation is very slow or absent in very acid soils and Mn^{2+} predominates; organic matter can reduce the higher oxides. In alkaline soils, the divalent form nearly disappears: bacterial oxidation is rapid and reduction by organic matter is slow. In well-aerated soils with a pH of more than 5.5, soil microorganisms can oxidize the divalent form rapidly.

There are two main mechanisms involved in the retention of Mn by soil. Firstly through cation exchange reactions, Mn ions and the charge surface of soil particles form Mn oxides, hydroxides and oxyhydroxides, which in turn form adsorption sites for other metals. Secondly, Mn can be adsorbed to other oxides, hydroxides and oxyhydroxides through ligand exchange reactions. When soil solution becomes saturated, these Mn oxides, hydroxides and oxyhydroxides can precipitate into a mineral phase and act as a new surface to which other substances can absorb (Evans, 1989).

The tendency of soluble Mn compounds adsorbed to soils depends on CEC and the organic composition of soil (ATSDR, 2000). Soil adsorption constants (the ratio of concentration in soil to the concentration in water) for Mn^{2+} span five order of magnitude increasing as a function of the organic content and the ion exchange capacity of the soil; thus, adsorption may be highly variable (Baes and Sharp, 1983). In some cases, adsorption of Mn to soils may not be a readily reversible process. At low concentrations, Mn can be fixed by clays and will not be released into solution (Reddy and Perkins, 1976). At high concentration, Mn can be desorbed by ion exchange mechanisms with other ion in solution (Rai et al., 1986). Mn mechanism in soil is shown in Figure 2.4.

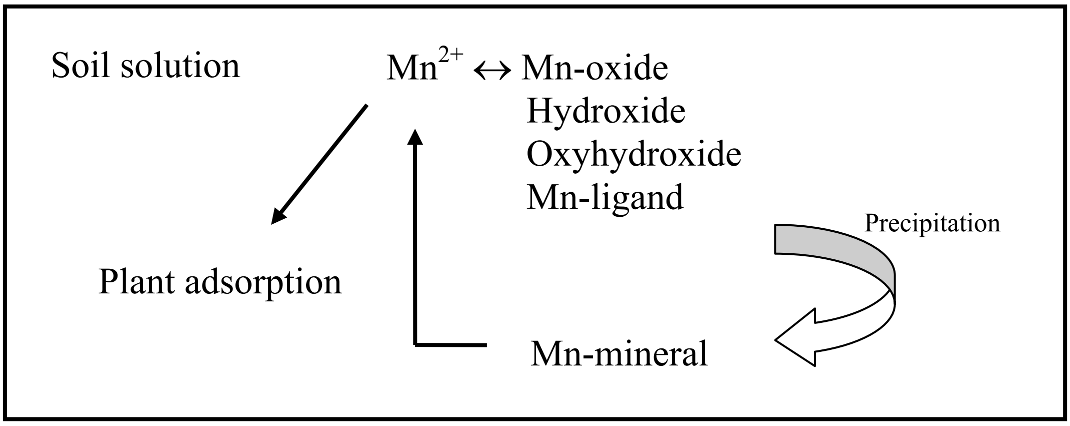


Figure 2.4 Mn fate and mechanism in soil (Adapted from ATSDR, 2000)

(4) Manganese uptaken by plant

Terrestrial plant species vary a great deal in their ability to accumulate Mn. The total concentration of Mn in soils is generally less important to plants than the availability of Mn, which is determined by pH, CEC, concentration of other cations, organic content, temperature and microbial activity.

Plants mainly absorb Mn in divalent state. Differences in plant uptake can be explained in part by differences in ability of plants to bring about the dissolution of oxidized Mn (Stokes et al., 1988). The availability soil Mn is closely influenced by the activity of microorganisms that can alter pH and redox potentials. Reducing the soil pH or soil aeration by flooding or compaction favors the reduction of Mn to the Mn^{2+} form and thereby increases its solubility and availability to plants. Heavy fertilization of acid soils without liming (particularly with materials containing chloride, nitrate, or sulfates) may increase availability and solubility. Under some conditions of pH and aeration, the addition of organic matters to soils can increase the chemical reduction of Mn and its uptake by plants. Furthermore, the application of chelating agent significantly reduced the uptake of Mn in roots, stems and leaves of okra (*Abelmoschus esculentus*) at Mn concentrations of 500 and 1,000 mg/kg (Denduluri, 1994).

Nitrogen applications consistently reduce the availability of Mn in soils. Organic materials associate with a high pH can produce organic complexes of Mn^{2+} leading to insufficient available Mn for susceptible plants such as peas or cereals.

Toxicity of Mn in plants may occur in soils containing Mn level exceeding 1,000 mg/kg dry weight (dw), this generally occurs in very acid soils and can remedy by liming (IPCS, 1981). The Mn contents in soils are little biological significance, since only a small amount is present in available form.

2.2.2 Environmental fates of copper

(1) Copper in air

Cu is released to the atmosphere in form of particulate matter or adsorbed to particulate matter. Few data are available regarding the chemical forms of Cu in atmosphere and their transformations. In the absence of specific information, it is generally assumed that metals of anthropogenic origin, Cu exists in the atmosphere as oxides because metallic species are readily attacked by atmospheric oxidants.

The particulate matter is removed from atmosphere by gravitational settling (bulk deposition), dry deposition (inertial impaction characterize by a deposition velocity), in clouds scavenging (attachment of particles by droplets within clouds), and wash out (collision and capture of particles by falling raindrops below clouds) (Schroeder et al., 1987). Removal rate and distance traveled from the source will depend on a number of factors, including source characteristics, particle size, turbulence, and wind velocity.

Gravitational settling governs the removal of large particles with mass median aerodynamic (MMA) diameters of >5 µm, whereas smaller particles are removed by the other forms of dry and wet deposition. The importance of wet to dry deposition generally increases with decreasing particle size (IPCS, 1998).

Soils containing Cu transport to atmosphere by wind blowing and Cu in water can enter to air from spraying.

(2) Copper in water

Cu discharged into waterway is in form of particulate matter and settle out. In water column and in sediment, Cu adsorbs to organic matter, manganese oxide, hydrous iron and clay. A fraction of Cu is significantly adsorbed within the first hour of introduction and equilibrium is normally reached within 24 hours (Harrison and Bishop, 1984).

Generally, species of Cu in water are present in the form of Cu^+ and Cu^{2+}. The Cu^+ is unstable in aqueous solution, tending to disproportionate to Cu^{2+} and Cu metal unless a stabilizing ligand is present (EPA, 1979; Kust, 1978). The only Cu^+ compounds stable in water are insoluble ones such as Cu_2S, CuCN, and CuF. Cu in its Cu^{2+} state forms is coordination compound or complexes with both inorganic and organic ligands. Ammonia and chloride ions are examples of species that form stable ligands with Cu. Cu also forms stable complexes with organic ligands such as humic acid, binding to $–NH_2$ and –SH groups, and to a lesser extent, with –OH groups (ATSDR, 2004).

The concentration of dissolved Cu depends on factors includes; pH, oxidation-reduction potential of water, presence of competing cations (Ca^{2+}, Fe^{2+}, Mg^{2+}), salts (OH^-, S^{2-}, $PO_4^{3-,}$ CO_3^{2-}), and anions of insoluble cupric–organic and inorganic complexing agent. If the combination of a particular anion with Cu formed an insoluble salt, precipitation of that salt will occur. The most significant precipitate formed in fresh surface waters is malachite ($Cu_2[OH]_2CO_3$). Other important precipitates are $Cu(OH)_2$ and azurite ($Cu_3[OH]_2[CO_3]_2$) (EPA, 1979). The combined processes of complexation, adsorption, and precipitation control the level of free Cu^{2+} in water, these processes reduce the free Cu^{2+} concentration to extremely low values.

As a result of the aforementioned physico-chemical processes, copper in water may be dissolved or associated with colloidal or particulate matter. Copper in particulate form includes precipitates, insoluble organic complexes, and copper adsorbed to clay and other mineral solids. McIlroy et al. (1986), studied using suspended solids from the Flint River in Michigan, found that the fraction of adsorbed copper increased sharply with pH, reaching a maximum at a pH of 5.5–7.5 (McIlroy et al. 1986).

In sediment, Cu is generally associated with mineral matter or tightly bound to organic material (Kennish 1998). As is common when a metal is associated with organic matter, Cu generally is associated with fine, as opposed to coarse, sediment. Furthermore, anaerobic sediments are known to be the main reservoir of Cu. Under anaerobic conditions, Cu^{2+} salts will reduce to Cu^+ salts. The precipitation of cupric sulfide and the formation of copper bisulfide and/or polysulfide complexes determine Cu's behavior in these sediments (Davies-Colley et al., 1985). In the more common case where the free sulfide concentration is low due to the controlling coexistence of iron oxide and sulfide, anaerobic sediment acts as a sinks for Cu, that is, the Cu is removed from water and held in the sediment as an insoluble cuprous sulfide. However, in the unusual situation where the free sulfide concentration is high, soluble cuprous sulfide complexes may form, and the Cu concentration in sediment pore water may then be high (ATSDR, 2004). Therefore, sediment is a large reservoir of the Cu. The transportation of Cu in water fate is shown in Figure 2.5

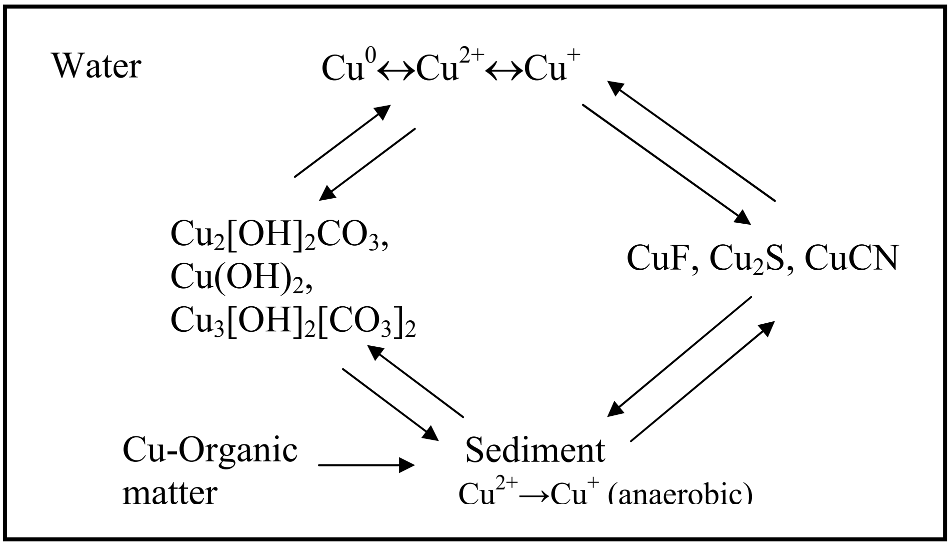


Figure 2.5 Transportation of Cu in water fate (Adapted from ATSDR, 2004)

(3) Copper in soils

Understanding the transport and fate of Cu and its compounds in soil is important because these compartments tend to be large reservoirs of Cu and could have an impact on human exposures of this pollutant. The movement of Cu in soil is determined by the physical and chemical interactions of Cu with the soil component. The predominated Cu in soil environments is Cu^{2+}. This cation can be adsorbed onto negatively charged soil minerals such as clays and strongly adsorbed on metal oxides, particularly Mn and Fe oxide (Dixon and Weed, 1989). Cu^{2+} is known to form very stable inner-sphere complexes with Fe and Al hydroxyl surface group. The sorbed Cu may eventually diffuse and irreversibly bind into internal sorption sites of metal oxide (Jenne, 1998). The Cu ion is subject to cation exchange reactions in the soil environment, mainly controlled by the presence of clay minerals and pH. Lindsay (1979) estimated the soil-Cu thermodynamic equilibrium constant (K°) to be $10^{2.80}$. Therefore, in a typical soil, if pH is known, the activity of Cu^{2+} is given by the following relationship;

$$\log Cu^{2+} = 2.8\text{-}2pH$$

This makes the soil-Cu much less soluble than most of the other carbonates and oxides of Cu.

Most of Cu minerals are unstable in the soil environments. However, under some conditions, Cu can form several relatively soluble sulfate, carbonate, hydroxide based minerals such as chalcocyanite ($CuSO_4$), brochantite ($Cu_4(OH)_6SO_4$) and crystalline forms of Cu carbonate ($CuCO_3$) and Cu hydroxide ($Cu(OH)_2$). But it is unlikely that minerals will form or be stable soil environment because of their high solubility (Lindsay, 1979).

The solubility of Cu in soils depends on pH and oxidation-reduction potential. In the reducing environment, solubility is low, possibly due to the formation of sulfides (Hermann and Neumann-Mahlkau, 1985). The greatest amount of leaching of Cu occurs from sandy soils, compare with clays and peat, whereas acidic conditions particularly acid rain (pH 2.8-4.2) favor Cu leachability (IPCS, 1998). The form of Cu in soil is determined by measuring the extractability of Cu with solvents. Extractability is a function of the nature of soil and the form of Cu deposited in soil. If a labile form of Cu is applied, binding to organic and inorganic ligands may occur, as well as other transformations. On the other hand, if mineral form is deposited, it would be unavailable for binding (ATSDR, 2004). In study of Cu partitioning in soils, sequential extractions were used to define six soil fractions in decreasing order of Cu availability: water soluble > exchangeable > carbonate > Fe-Mn oxide > organic > residual (Ma and Rao, 1997). The results of this study showed that the distribution of Cu in these six soil fractions differed depending on the total concentration of Cu in the soil. As the Cu concentration increased above 240 mg/kg, between 69 and 74.4% of the total Cu was found in the water soluble, carbonate, Fe-Mn oxide, and organic fractions. In relatively uncontaminated soils (<240 mg/kg copper), between 97.6 and 99.6% of the Cu was found to be associated with the residual fraction.

Although Cu^{2+} dominates the solid solution chemistry of typical soils, it is possible to reduce to Cu^{+} when soil conditions favor it. The reduction of Cu^{+} to elemental Cu (Cu^{0}) is unlikely in most soils since typically soil redox potentials are not lower than -400 mV and the redox potential of Cu^{+}/ Cu^{0} couple is 525 mV. The redox reaction of Cu^{2+} to Cu^{+} is:

$$Cu^{2+} + e^{-} \leftrightarrow Cu^{2+}$$

Where log K° = 2.62

Therefore, when the activities of these two species are equal, log of soil redox potential will be equal to log K° = 2.62 or + 155 mV. Thus under reducing (waterlogged anaerobic) soil conditions, cuprous ferrite ($Cu_2Fe_2O_4$) may form and be stable. Under extremely reduced and acidic soil condition, reduced Fe minerals will become dominant and may lead o formation of metallic (Cu^{0}), which can coexist with cuprous ferrite and iron minerals like magnetite (Fe_2O_4). In sulfide rich soil/sediment environments, the formation of copper sulfide minerals is possible. Chalcocite and even covellite may form, and coexist with $Cu_2Fe_2O_4$ when the combined of pH and soil redox potential are ≤ 4.73 (Lindsay, 1979).

The chemistry of soil solution is dominated by Cu^{2+}, hydroxide, carbonate and sulfate complexes. In organic soil, stable Cu chelates are readily formed with humic substances (humic and fulvic acids) and may dominate the solid-solution chemistry. Soil Cu-organic matter chelates are known to significantly reduce Cu bioavailability and mobility in organic soils. This is due to the formation of stable Cu-organic matter chelates using carbon and nitrogen groups like $–COO^{-}$, $-NH_2$ and other aliphatic and aromatic electron rich groups found in organic matter structures. For example, insoluble peat and humic acids strongly chelate Cu^{2+} by forming a square planar arrangement with two carboxylate and two aliphatic N-ligands (MacCarthy et al., 1990). Soil microorganisms can affect the absorption of Cu in soils by the assimilation and uptake of Cu. However, it does not know the rate of uptake and absorption capacity of the microorganisms for Cu compare with the binding capacity and affinities of Cu by organic matter in soils (Rieuwerts et al., 1998). There have reports that Cu binds strongly to soils with high organic content and the distribution of Cu in soil solution is less affected by changes in pH (within the range of pH normally encountered in the environment). When the amount of organic matter is low, the mineral content or Fe, Mn, and Al oxides become important in determining the adsorption of copper (Rieuwerts et al., 1998). In laboratory study of adsorption and leaching of Cu in soil column of widely different characteristics, Cu eluted in $CaCl_2$ (leaching solution) much more slowly and low quantities from a low pH and high pH mineral soils and not at all from peat soil, which contained the greatest amount of organic matter (Tyler and McBride, 1982). The study of pH-dependent adsorption of Cu in two mineral soils (silty clay loam (0.5 g/kg organic dry weight) and sandy clay (1.6 g/kg organic dry weight)) and two soils containing considerable organic matter (loamy sand (20.5 g/kg organic dry weight) and silt loam (42.5 g/kg organic dry weight)) was investigated by Elliott et al. (1986). Adsorption increase with pH and Cu much more strongly retained. The absorption was reduced after removal of organic matter demonstrated the importance of organic matter in Cu binding. Wu et al. (1999) studied clay soils, they observed preferential Cu binding to organic matter, but found higher binding affinities to fine (< 0.2 μm) clay fractions once the organic matter had been removed.

In the absence of significant of amounts of natural organic matter and in natural to alkaline conditions, the $Cu(OH)_2$ species will predominate in solution. Normal soils usually contain significant amount of carbonates and sulfates. Therefore, $CuCO_3$ and $CuSO_4$ as well as Cu^{2+} and $Cu(OH)_2$, species will dominate (95%) the soil solution, depending on the pH and the activities of two counter anions. For example, in acidic soil with free sulfate ions ($\sim 10^{-2.4}$M), the Cu SO_4/Cu^{2+} may be near 1. Note that in water solution, Cu^{2+} is hydrate with four to six water molecules, for example, $Cu(H_2O)_4^{2+}$, a favored complex. These complexes predominated at pH < 6 in the absence of any competing ligands. In alkaline soils with normally elevated CO_2 ($\sim 10^{-2.4}$atm), the $CuCO_3/Cu(OH)_2$ may be near 1 (Lindsay, 1979). In both cases, it can be assumed that the Cu-organic complexes or chelates are negligible. Cu ions can form complexes with N-based ligands like ammonia (NH_3), nitrate (NO3-), phosphate (HPO_4^{2-}, $H_2PO_4^-$), and chloride (Cl^-) ions in soil solutions. But their contributions to the total Cu species in typical soils solutions are very small (ATSDR, 2004). Figure 2.6 shows the transportation of Cu in soil environment.

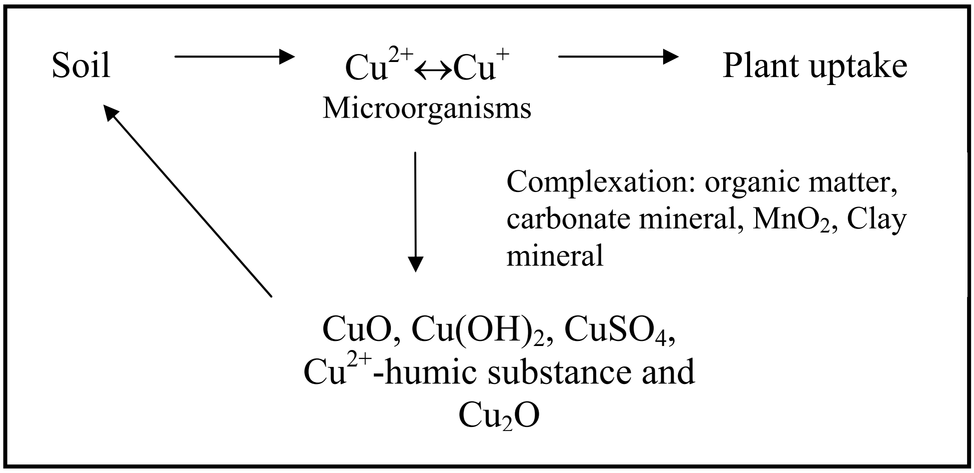


Figure 2.6 Fate and transportation of Cu in soil (Adapted from ATSDR, 2004)

(4) Copper uptaken by plant

Cu is an essential nutrient in plant metabolism. Therefore, uptake of Cu from soil in plants through the roots is a natural and necessary process (Clemens, 2001). The uptake of Cu into plants is dependent on the concentration and bioavailability of Cu in soils. The bioavailability of Cu is determined largely by the equilibrium between Cu bound to soil components and Cu in soil solution. Cu binding in soils, this is determined by Cu concentrations in soil, soil type, soil components, pH, oxidation-reduction potential in the soil, and concentrations of other cations and anions in the soil (Rieuwerts et al., 1998). Other factors include root surface area, plant genotype, stage of plant growth, weather conditions, interaction with other nutrients in the soil and water table (Gupta, 1979). When Cu is applied to soils, it is strongly bound to clay minerals. Exchangeable Cu is held much more tightly than other cations, and is not readily available to plant. Organic matter also binds Cu, so the more organic matter and clay that a soil has, the greater the potential for Cu adsorption will be. In addition, increasing soil pH reduces Cu availability to plants, allowing greater soil Cu accumulation without subsequent plant toxicity in high pH soil. Because of its strong binding, Cu leaches very little, and accumulates in soil surface. Therefore, decreasing pH can increase copper availability (ATSDR, 2004).

Liming is another factor that affects Cu uptake. For example, liming acidic soils has been shown to increase uptake in hay, but to decrease Cu uptake in wheat

(Gupta, 1979). However, the effect that liming has on increasing soil pH does not appear to be the overriding mechanism behind the changes in Cu uptake in plants, even though there is evidence that the addition of lime to soil increase the pH to 7 or 8 reduces copper availability to some plants (IPCS, 1998). This is evidenced by the fact that changes in pH (5.4-8.0) have been found to have little effect on Cu concentrations in plant tissues (Gupta, 1979).

2.3 Ecological Toxicities

Mn and Cu are the essential elements for all living organisms. They are regular part of living bodies to work properly, including, components in many enzymes that are used for the metabolisms and essential for normal growth. The serious systemic health effects can present as the result of high accumulation of dietary of these elements in living bodies.

2.3.1 Ecological toxicity of manganese

(1) Effects to animals

For some animals, the lethal dose is quite low, which means that they have little chance to survive even smaller doses of Mn when these exceed the essential dose. Mn substances can cause lung, liver and vascular disturbances, declines in blood pressure, failure in development of animal fetuses and brain damage.

In study of platyhelminths, Mn in divalent form was neurotoxic, blocking presynaptic release of dopamine, leading to irreversible damage to the dopaminergic cells (Palladini et al., 1980). Concentration of Mn (added as manganese sulfate or manganese chloride) of 100-1,000 mg/liter immediately induced screw-like movements of the worms due to dopaminergic over stimulation. This was followed by death within 24-48 hours (IPCS, 2004).

Furthermore, when Mn uptake takes place through the skin it can cause tremors and coordination failures. Finally, laboratory tests with test animals have shown that severe Mn poisoning should even be able to cause tumor development with animals (IPCS, 1981).

(2) Effect to plants

Mn is required for healthy plant growth. Plant roots have a Mn absorption mechanism that provides sufficient Mn for healthy growth in most soils. In some acid soils, solution Mn levels may reach very high levels. Many plants will absorb more Mn than they require internally. If they do not have internal mechanism to control cellular Mn concentrations, toxicity effects occur.

Symptoms of Mn toxicity to plants vary widely with species include marginal chloroses, necrotic lesions, and distorted development of leaves. The toxic Mn concentration in crop plant tissues vary widely, with critical values ranging from 100-5,000 mg/kg. Symptoms of toxicity are rarely seen in grapevines, there show black spots on leaf blades, shoots and bunch stems.

Factors effecting Mn tolerance in plants include genotype, silicon concentration, temperature, light intensity, physiological leaf age, microbial activity, and the characteristic of rhizosphere (IPCS, 1981).

(3) Effect to human

Mn enters to human body by inhalation and gastrointestinal system. Mn is absorbed in small intestines and the absorption process is slow. Excess Mn is excreted in bile and pancreatic secretion. Only small amounts are excreted in urine.

Excess Mn interferes with the absorption of dietary iron. Long-term exposure to excess levels may result in iron-deficiency anemia. Increased Mn intake impairs the activity of Cu metallo-enzymes. Symptoms of toxicity mimic those of Parkinson's disease (tremors, stiff muscles) and excessive Mn intake can cause hypertension in patients older than 40. Significant rises in Mn concentrations have been found in patients with severe hepatitis and posthepatic cirrhosis, in dialysis patients and in patients suffering heart attacks. Symptoms of increased Mn levels are present include:

- Psychiatric illnesses
- Mental confusion
- Impaired memory
- Loss of appetite
- Mask-like facial expression and monotonous voice

- Spastic gait
- Neurological problems (similar to Parkinson's disease) (Blaurock-Busch, 1997)

The upper limit of safety for Mn established by the Food and Nutrition Board of the Institute of Medicine is approximately 11 mg daily for adult (Institute of Medicine, 2001). The Manganese Tolerable Upper Intake Levels are shown in Table 2.3.

Table 2.3 Mn Tolerable Upper Intake Levels

Age (years)	**Manganese Tolerable Upper Intake Levels (mg/kg/d)**
Infants, 0.0-0.5	ND
Infants, 0.6-1.0	ND
1-3	2
4-8	3
Males, Females	
9-13	6
14-18	9
>18	11
Pregnancy, Lactation	
<18	9
19-50	11

Source: Adapted from Institute of Medicine (2001)

ND = Not determination

2.3.2 Ecological toxicity of copper

(1) Effect to animals

Cu can become toxic to organisms when concentrations are too high. The toxicity of Cu varies widely between species. The more soluble salts (Cu sulfate ($CuSO_4$), Cu chloride ($CuCl_2$)) are generally more toxic than the less soluble salts (Cu hydroxide ($Cu(OH)_2$), Cu oxide (CuO)). Death is preceded by gastric haemorrhage, tachycardia, hypotension, haemolytic crisis, convulsion, and paralysis.

The study showed that, rat given up to 305 mg Cu/kg per day orally in the diet as $CuSO_4$ for 15 days showed alteration in blood biochemistry and haematology (particularly anemia) and adverse effects on the liver, kidney, and lungs. The no-observed-effect level (NOEL) in this study was 23 mg Cu/kg body weight per day.

Soils contain significant Cu residues have been observed to have few earthworms (Van Zwieten et al., 2004). It has been noted that earthworms exhibit chronic toxic responses at relatively low levels of Cu with concentrations < 4-16 mg/kg (Helling et al., 2000). The enchytraeid worm (*Cognettia sphagnetorum*) was shown to actively avoid Cu contaminated soil (Salminen and Haimi, 2001).

Studies of neurotoxicity have not shown effects on behavior but neurochemical changes have been reported after oral administration of 20-40 mg Cu/kg body weight per day (IPCS, 1998).

(2) Effect to plants

Cu is an essential element for various metabolic processes because it is required only in trace amounts, Cu becomes toxic at high concentration. Cu is adsorbed by plant roots as Cu^{2+}.

Cu is known to damage cell membranes by binding to the sulphydryl groups of membrane proteins and by inducing lipid peroxidation. In non-tolerant plants, inhibition of root elongation and damage of root cell membranes are the immediate response to high Cu levels (Wainwright and Woolhouse, 1977). Moreover, excessive Cu reduced plant root length, root dry weight, total dry weight, root to shoot ratio, leaf area, and specific leaf area in plants (IPCS, 1998).

(3) Effect to human

Cu becomes toxic to human body when intake in a large amount. The lower limit of acceptable range of oral intake is 20 μg Cu/kg body weight per day. Cu may be adsorbed by both the stomach and small intestinal mucosa, with absorbed by the small intestine. The average level of Cu stored in the body is from 50 to 120 mg, with most of this in the liver (IPCS, 1998).

Excessive intake of Cu can cause abdominal pain and cramps, nausea, diarrhea, vomiting, and liver damage. In addition, some experts believe that elevated

Cu levels, especially when zinc levels are also low, may be a contributing factor in many medical conditions including schizophrenia, hypertension, stuttering, autism, fatigue, muscle and joint pain, headaches, hair loss in woman, childhood hyperactivity, depression, insomnia, senility, hallucination, paranoia, and premenstrual syndrome.

The toxic effects of high tissue levels of Cu are seen in patients with Wilson's disease, a genetic disorder characterize by Cu accumulation in various organs due to the inadequate synthesis of ceruloplasmin (the protein that transports Cu through the blood) by liver. Wilson's disease primarily affects the liver, kidney, and brain causing degenerative physiological changes, including cirrhosis of liver, muscular rigidity and spastic construction, and emotional disturbance, these are fatal if untreated. The treatment of Wilson's disease involves avoidance of food rich in Cu and any supplements containing Cu (Groff et al., 1995).

In 2001, the Institute of Medicine at the National Academy of Sciences established the following Tolerable Upper Intake Levels for Cu is shown in Table 2.4. The upper limit of safety establish for Cu is approximately 900 μg/kg/d for adult.

Table 2.4 Tolerable Upper Intake Levels for Cu

Age (years)	**Tolerable Upper Intake Levels for copper (μg/kg/d)**
0-1	ND
1-3	1,000
4-8	3,000
Males, Females	
9-13	5,000
14-18	8,000
>18	10,000
Pregnancy (<18)	8,000
Pregnancy (>18)	10,000
Lactation (<18)	8,000
Lactation (>18)	10,000

Source: Adapted from Institute of Medicine (2001)

ND = Not determination

2.4 Mn and Cu Contaminations in Agricultural Soils

Mn and Cu enter to agroecosystem through both natural and anthropogenic processes. Nature soil receives these elements from its parent materials as background levels. In soil, Mn and Cu are generally ranged from 500-900 mg/kg (IPCS, 1981) and 2-250 mg/kg (IPCS, 1998), respectively. Agricultural practices are very important non point sources of these metals that make significant contribution to their concentrations in soils and lead to contaminations in other media. Agricultural land receives the anthropogenic inputs of Mn and Cu from the applications of pesticide, fertilizer, farmyard manure, lime material and organic compost. The processes unless the directly inputs that contribute these elements to soil ecosystem include washing or leaching from plant parts and importantly atmospheric deposition (wet and dry process) (Alloway, 1995).

Applications of Mn and Cu as the essential elements for plant growth are common practices for correcting deficiencies in agriculture especially for fruiting of grapevines. Mn and Cu containing in fertilizers include $MnSO_4$, $CuSO_4$ and Mn-chelate (Mn-EDTA). If Mn and Cu are required, foliar nutrient spraying of these compounds are normally used. Furthermore, farmyard manures and lime materials are well established present the high concentration of these elements. The typical concentrations of Mn and Cu in fertilizers, farmyard manures, limes, and composts are shown in Table 2.5, where it can be seen that phosphate fertilizers can be the important source (Alloway, 1995).

Table 2.5 The typical concentrations of Mn and Cu in fertilizers, farmyard manures, limes, and composts (mg/kg)

	Phosphate fertilizers	Nitrate fertilizers	Farmyard manure	Lime	Composted refuse
Copper	1-300	-	2-172	2-125	13-3580
Manganese	40-2,000	-	30-969	40-1,200	-

Source: Alloway (1995)

Many pesticides including fungicides, herbicides and insecticide are the important source of Mn and Cu contaminate soil environment due to the repeated use of these compounds for curing or preventing diseases of many fruit, importantly grapes. Pesticides contain with Mn and Cu as a part of active ingredient. Especially fungicides, for example, mancozeb (manganese ethylenebis(dithicarbamate) polymeric complex with zinc salt) and maneb are fungicide in group of ethylene dithiocarbamates, these compounds consist of Mn salt as an active ingredient and have low soil persistence with half-life less than 2 and 8 days in aerobic and anaerobic soil. Although this compounds are non-persistence in environment and quickly degraded into other compounds but metals in their molecules are non-biodegradable and tend to persist for long period of time. He et al. (2005) observed that, use of maneb and mancozeb had annual loading rates of Mn approximately 0.4-0.5 kg/ha to soils, recommended in 1964-1975. Furthermore, Cu has been well established as an effective protectant fungicide. Cu ion releases to denature proteins and enzymes within fugal spores. Spores actively accumulate Cu and germination is inhibited even at low concentration. Cu fungicides widely use in viticultural activities include copper hydroxide ($Cu(OH)_2$), copper sulfate ($CuSO_4$) and copper oxychloride ($CuCl_2{\cdot}3Cu(OH)_2$). He et al. (2005) proposed that spraying Cu-containing chemicals added 1-2 kg/ha/year to soils.

Gimeno-Garcia et al. (1996) were measured heavy metal contents in fertilizer and pesticide, found that copper sulfate (25% Cu) contained 255×10^3 mg/kg of Cu, iron sulfate (18.5% Fe) contained 0.3 mg/kg of Cu and 220 mg/kg of Mn and 70% propineb contained 15 mg/kg of Mn.

The repeated use of metal based agrochemicals has led to long term accumulation of Mn and Cu in soil. There had researches studied of Mn and Cu contents in vineyard soils due to the applications of pesticides and fertilizers. Rusjan et al. (2006) proposed that Mn and Cu in soils in vine growing region of Slovenia were averaged 1,251 mg/kg and 7 mg/kg. In Victorian vine growing, total Cu concentration in some vineyard soil was increased to 250 mg/kg compared with the background values, approximately 10 mg/kg (Pietrzak and McPhail, 2004).

Factors affecting metal in soil include;

(1) pH

Soil pH reaction is an indication of the acidity or alkalinity of soil and measure in pH unit. Soil pH is defined as the negative logarithm of the hydrogen ion concentration. The pH scale ranges from 0-14, with pH 7 as the neutral point. As the amount of hydrogen ions in soil increases, soil pH decreases that becoming more acidic. From pH 7 to 0 the soil is increasingly more acidic and from pH 7 to 14 the soil is increasing more alkaline or basic (Bailey, 1996).

The effect of soil pH is on the solubility of minerals or metals. Before metals can be uptake by plants, it must be dissolved in soil solution. Most metals or mineral are more soluble or available in acid soils than in neutral or slightly alkaline soils. Mn and Cu are available for plant uptake at pH below 5.0, although their availability can be excessive and thus toxic in more acidic condition. In alkaline soil, metals are less available and symptoms of deficiency can develop. The availability of minerals which affected by pH is presented in Figure 2.7. Black areas present relative availability (Bailey, 1996).

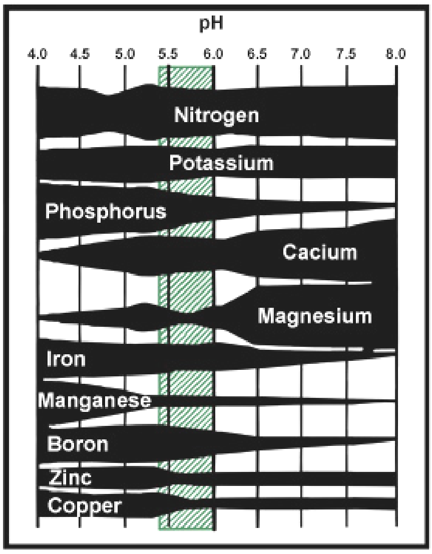


Figure 2.7 The availability of minerals affected by pH (Bailey, 1996)

The soil pH can influence on plant growth by its effect on activity of microorganisms. Bacteria that compose soil organic matter are hindered in strong acid soils. That prevents organic matter from breaking down, affecting in an accumulation of organic matter and the tie up of metal that are held in organic matter (Smith et al., 1994).

(2) Cation exchange capacity (CEC)

Cation exchange capacity (CEC) is defined as the degree to which a soil can adsorb and exchange cation. In solution, dissolved substances exist as charged particles, positive or negative charges. Soil particle and organic matter have negative charge (NO^{3-}, CO_3^{2-}, OH^-) on their surfaces. Mineral cation (Mn^{2+}, Cu^{2+}, Na^+) can adsorb to the negative surface charges or the inorganic and organic soil particles. The larger capacity to adsorb cations defines as the larger CEC. Soil components vary in the ability to attract and hold ions. Organic materials such as humus fulvic acid and clay particles are capable of adsorbing ions to a much larger degree compared with sand, which have zero CEC (Pennisi and Thomas, 2005). Figure 2.8 shows the reaction between cationic charges and negative charges. Once adsorbed, these minerals are not easily lost when the soils are leached by water and they provide minerals available to plant roots.

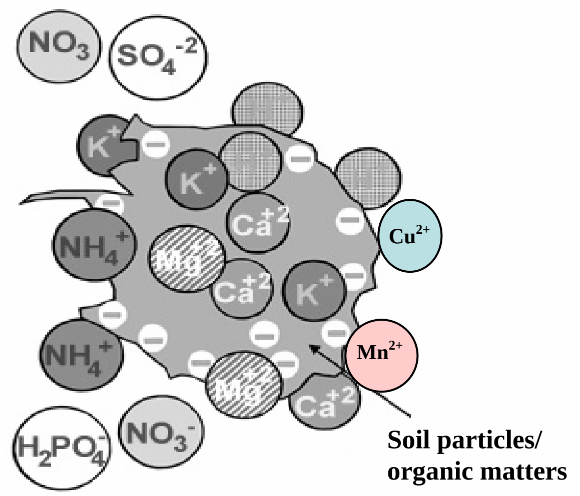


Figure 2.8 The reaction of soil materials (Pennisi and Thomas, 2005)

(3) Soil organic matter

Soil organic matter (OM) represents the remains of roots, plant material and soil organisms in various stages of decomposition and synthesis and is variable in composition. Though occurring in relatively small amounts in soils, OM has a major influence on soil aggregation, mineral reserve and its availability and biological activities. Soil organic matters include humus or humic acid and fulvic acid, surface of these compounds have negative charges. Cations can be adsorbed to the surfaces and hold ions to form organic complexes or organic ligands by ionic forces, van der Waal forcs or hydrogen forces (Pennisi and Thomas, 2005).

Humus strongly binds metals to its negatively charged sites, providing major reserve in top soils. In alkaline soils high in organic matter, binding can cause deficiencies of metals. Toxicity of metals can occur with high organic matter in acidic condition.

(4) Oxidation-reduction potential

Oxidation-reduction potential (Redox potential) is an electrical measurement that shows the tendency of soil solution to transfer electrons to or from reference electrode. Changes in redox potential are the result of the combined effects between many chemical, physical and biological processes in soil. Redox potential is, together with pH, a driving variable for speciation of metals (Salomons and Stigliani, 1995).

Soil oxidation and reduction depend on soil respiration, diffusion of oxygen and carbon dioxide in soil and on changes in biochemistry of system (Nemecek et al., 1990). Redox potential was studied mostly in anaerobic soils, where the high moisture content decreased the rate and amount of O_2 diffusing into soil (Bailey and Beuchamp, 1973; Savant and Ellis, 1964). In anaerobic soils, redox potential was shown to be negatively correlated with microbial activity (Kralova et al., 1992; Snakin and Dubinin, 1980). Low redox potential developed with increased soil moisture content because of partial or complete displacement of O_2 from soil and rapid consumption of O_2 by soil microbes (Savant and Ellis, 1964). Redox potential in anaerobic condition was shown to be pH dependent (Yli et al., 1999; Dragun, 1993 and van Breemen, 1987). The alkaline and pH of soil tend to increase with chemical reduction, and to decrease with oxidation (van Breemen, 1987).

Redox potential was rarely studied in aerobic soils, where O_2 concentration is relatively high. It was found that redox potential can be affected by microbial activity in aerobic soils which it serve as a good indicator of nitrogen loss during temporarily anaerobic conditions in arable soils (Reddy and Patrick, 1975). Volk (1993) showed that in arable soils moisture indirectly decreased redox potential by increasing bacterial activity. In aerobic field conditions, where microbial activity is high, redox potential will be negatively correlated with nitrification, because of microbial consumption of soil O_2. While under different condition, where microbial activity is limited by physical and chemical characteristics in soils, redox potential and nitrification will both be positively but indirectly correlated via their relationship with those limiting characteristics such as porosity or soil moisture.

For example, Mn reduction from insoluble oxidized forms to slightly soluble reduced forms occurs readily when an aerated soil is waterlogged, together with the disappearance of O_2 and NO_3^- (Patrick and Turner, 1968). The solubility of Cu in soils depends on pH and oxidation-reduction potential. In the reducing environment, solubility is low, possibly due to the formation of sulfides (Hermann and Neumann-Mahlkau, 1985). Cu^{2+} is dominates of the solid solution chemistry of typical soils, it is possible to reduce to Cu^+ when soil conditions favor it. The reduction of Cu^+ to elemental Cu (Cu^0) is unlikely in most soils since typically soil redox potentials are not lower than -400 mV and the redox potential of Cu^+/ Cu^0 couple is 525 mV (Lindsay, 1979).

(5) Soil texture

Soil texture describes the proportion of sand, silt and clay particles found in the soil. These proportions determine:

- The size of the pore spaces between the soil particles and the air, and to a large extent,
- The moisture and metals holding capacity of the soil.

Soil particles less than 2 mm in size are classified into threes sizes: sand (largest), silt, and clay (smallest). Particles over 2 mm are coarse fragments, gravel, rocks and woody debris. The coarse particles and debris have little impact on growth and moisture storage characteristics of the soil, although they can be a problem during construction and later use of the finished surface.

Soils are classified according to the ratios of these different sized particles. For example, soils classed as “sandy” soils are more than 50-55% sand, whereas “clay” soils have a clay content of 20% or more. “Loam” is an intermediate classification with all three particle sizes well represented. The sand component strengthens soil and improves drainage, while the finer clay and silt particles hold metals and make them available to plant roots. Cation of metals (Mn^{2+}, Cu^{2+}, Na^{+}) will strongly bind with the surface of negative charges of clay. Loams also have a good balance between the larger pore spaces (aeration pores), which normally contain air, and the smaller pore spaces (capillary pores), which hold the moisture (Brady, 1984).

2.5 Good Agricultural Practices (GAPs)

Food safety concerns are increasing as once unheard of illness-causing microorganisms and toxic materials become more prevalent and as products previously considered safe cause an increasing number of illnesses. Produce, recently thought of as a safe products, have been identified as a cause of major food borne illness outbreaks in recent years.

Developing a safety plan helps food producers manage the safety component of operation by organizing the action steps identified as key to reducing risk from the microorganisms and toxic materials. Documenting for current practices and any changes over time allows for monitoring the safety of food product. The publication provides Good Agricultural Practices for developing a food safety plan. This term is not new and has begun to get a wide attention at the end of 1990s (UN FAO, 2007).

UN FAO give definition to GAPs, are the collection of principles applied for on-farm production and post-production processes, resulting in safe and healthy food and non-food agricultural products that taking into account economical, social and environmental sustainability. The production processes should play safe for producers and consumer, including the sufficient used of natural resources and establishment of sustainable agriculture systems which may reduce the contamination of hazardous materials in the environment.

GAPs can be applied to a wide range of farming systems and at difference scales which they are applied through sustainable agricultural methods, such as integrated pest management, integrated fertilizer management and conservation agriculture which there are four principles for GAPs (UN FAO, 2007):

- Maintain viable farming enterprises and contribute to sustainable livelihoods
- Economically and efficiently produce sufficient (food security), safe (food safety) and nutritious food (food quality)
- Sustain and enhance natural resources
- Meet cultural and social demands of society.

The objectives of GAPs

- In order to organize the appropriate technology of crop production
- In order to make a safe of the hygiene of human life
- To meet a basic need of high quality, save and free from pest on agricultural products
- Basic requirement for international trades on agricultural products

The component of GAPs

- Management on land
- Management of water source
- Management of crop production
- Pest management
- Harvesting, storage and manufacturing management
- Management on energy resource and waste product
- Safety, welfare and hygienic of the workers
- Wild lives, plant species and natural geographical feathers

The GAPs relate to soil

- Reducing erosion by wind and water through hedging and ditching
- Application of fertilizers and pesticides at appropriate moment and adequate doses and avoid run off the surface soil
- Avoid toxic materials for agricultural practices

- Maintaining or restoring soil organic content, manure application, use of grazing and crop rotation
- Reduce soil compaction issues such as avoiding using heavy mechanical devices
- Maintain soil structure (limiting heavy tillage practices)

The GAPs relate to water

- Practice schedule irrigation, with monitoring of plant needs and soil water reserve status to avoid water loss by drainage
- Prevent soil salinization by limiting water input to needs and recycling water whenever possible
- Avoid crop with high water requirements in a low availability region
- Avoid drainage and fertilizer run-off
- Maintain permanent soil covering, in particular in winter to avoid nitrogen run-off
- Manage carefully water table, by limiting heavy output of water

2.6 The Vineyard

The selected vineyard is located on Pak Chong District, Nakhonratchasima Province. The vineyard is situated on the edge of the Kao Yai National Park. This site is estimated to be one of Thailand's largest vineyards with 400 hectares plantations (or approximately 2,500 rais) and of which 80 hectares (or 500 rais) are used for vine growing with nineteenth year of operation. This site consists of wine grape approximately 64 hectares (400 rais), table grape 16 hectares (100 rais) and the trial activity 8 hectares (50 rais). The vineyard produces grapes for eaten fresh, grape juices and for wine-making (with full capacity of 1,000,000 bottles per year).

2.6.1 Vineyard description

The vineyard larger than several hectares is generally divided into blocks. This block presents a single variety. The areas of vine blocks are uncertainty which they depend on terrain of vineyard. The maximum vineyard productivity is attained when most of available sunlight is intercepted by grapevine leaves. Vine blocks are usually laid out in straight rows running in North-South direction to intercept the maximum amount of sunlight. However, plantations on sloping land provide as contour planting or straight rows across the slope regardless of compass orientation, this can reduce soil erosions. Vines in block are laid out with uniformly space vines and parallel in rows. Row spacing is about 2.5 m for training system and equipment accommodation. The spacing of vines in row is approximately 1.2-1.5 m. The vine plantations were presented in Figure 2.9.

Vines require trellises for growing. Trellises are used to support tendrils of vines and allow the vines to grow up from the ground that easy for training systems. For table grapes, the trellis are high cordon and wine grapes are low cordon systems. The trellis material is made from wire (Greenspan, 2006).

In vineyard, water sources are from two systems, underground water and reservoirs. The vineyard has water drainage systems flow in south-north direction which they use to collect water for non-flooding in vine blocks. In winter, the drainage systems are dry. Some irrigate to reservoirs which collect water for utility in vineyard and discharge to small public stream. Some drainage systems irrigate to small public stream directly. Figure 2.10 showed drainage systems in the study area.

The vineyard consists of three soil types which they were investigated by vineyard staffs. The types of soil are sandy loam, clay loam and loamy-stone. These present that the soils are poor fertile.

Most vines in this vineyard are *Vitis vinifera*. The varieties of vine were obtained from France, Spain, Italy and Germany which it is divided in two groups as below;

(1) Table grape

- Seedless: Marroo, Canner, Thompson, Flame, Loose perlette, Ruby and Crimson etc.
- Seeded: Cardinal, Black queen, Black opal and White Malaga etc.

(2) Wine grape

- Red wine: Shiraz, Tempranillo, Dornfelder, Carbinet Sauvignon and Pinot Noir etc.
- White wine: Chenin blance, Colombard and PB1 etc.

Figure 2.9 The plantation of vine in the study area, located at Pak Chong District

Figure 2.10 Water drainage system of the vineyard

Agricultural practices

In order to have maximum vine productivity and well growing grapes, the vines require attention for cultivation. Grapevines need fertilizing, weeding, insect and disease control, watering and proper training and pruning systems. The definitions of practices used in viticulture were shown below (Coombe and Dry, 1992);

Training

Training is the arrangement of plant parts spatially. This is done to develop the structure in order to optimize the utilization of sunlight, air movement for disease prevention and management practices (row for equipment). The training system is designed to encourage the production of new fruiting canes at specific positions on the vine-the arms. Proper training of grapevines is essential to maintain plant size, shape and productivity. Additionally, the practices can promote efficient and sustainable vineyard management practices.

Pruning

Pruning is the removal of plant parts to control size and form of grapevines, optimize the production potential of grapevines and maintain balance between vegetative growth and fruiting, such as selectively removal of unsuitable or extraneous canes, retaining a small number of good canes.

Agricultural practices relate to GAPs

The vineyard has been operated in manner of GAPs as shown below.

1) Pruning

- Wine grape: 2 times/year
- Table grape: 1 time/year

2) Fertilizing

Chemical fertilizers

Consist of soil applications and foliar sprays. Formulas such as: 15-15-15, 13-13-21, 8-24-24, 25-7-7 and 12-24-12, etc. Additions are about 50 g/vine/type of fertilizer.

- Wine grape: 12 times/year
- Table grape: 6 times/year

Manures

Additions are about 5 kg/vine.

- Wine grape: 2 times/year
- Table grape: 2 times/year

3) Clean up the vine

- Wine grape: 2 times/year
- Table grape: 1 time/year

4) Bud stimulation

- Wine grape: 2 times/year
- Table grape: 1 time/year

5) Pesticide spraying

Types of pesticide are recommended by Thailand Department of Agriculture.

Insecticide spraying

- Depend on the pest damage, climate conditions and crop growth stages
- Table grape more sensitive to insect pests than wine grape
- Pest investigation: every 2-3 day for wine grape and everyday for table grape

Fungicide spraying

In this study, we interested in fungicides because of these chemicals have Mn and Cu in their molecules. Mn and Cu contained fungicides widely used in this vineyard are 80 WP mancozeb (contain with 20% Mn-salt) and copper oxychloride 80 WG (contain with 50% Cu). These chemicals are used to control diseases which are downy mildew, powdery mildew, anthracnose and rust, etc. Information about use of these fungicides was not certain depend on disease outbreak, crop growth stages and climatic condition.

Wine grape was set into two crops mean that it was harvested 2 times/year. Table grape was set into one crop or harvest one time/year. The application was shown below;

- Copper oxychloride

Table grape was sprayed with these chemical 3-4 times/year approximately use 240 g/plot/year. Wine grape was estimated applications 4 times/year. Generally, wine grape was sprayed with high concentration than table grape estimated to highest application about 12 kg/year/plot.

- Mancozeb

Wine grape was sprayed with mancozeb estimated to be 8 times/year, in part of table grape, 3-4 times/year. The highest application was approximately 12 kg/plot/year for wine grape and not estimated for table grape (expected lower concentration than wine grape).

6) Weed control

- Mowing: 12 times/year for wine grape and 2 times/year for table grape
- Herbicide spraying: 6 times/year for wine grape and no information for table grape

7) Watering

- Wine grape: every 3-5 day
- Table grape: every 2 day

8) Other practices

- Wine grape: Tip pinching 5-6 times/year
- Table grape: Wrapping the fruit cluster when color of fruit change (green to brown/red) that approximate 80 day after pruning

9) Harvesting

- Grape vine: 100-120 day after pruning
- Table grape: 85-150 day after pruning

10) Residue of chemicals analysis

- Soil and water were analyzed next month after harvesting

2.6.2 The topographical features of vineyard

The vineyard is situated on the north of Khao Yai National Park with attitude of 14.3 degree north. This site is a plateau on 300 to 350 meters above sea level. The south is closed to hill and sloping down to the north. The adjacent areas are agricultural areas, residential areas and resort. The vineyard is identified on soil groups of 47 derived from the Department of Land Development, Ministry of Agriculture. This group is clay loam. This is well-drained soil which is often characterized by a sub-soil (layer below the topsoil) and has 2-20% of slope. The top soils are uniformly reddish-brown, red, yellow or brown. The subsoil (depth > 50 cm) is found as sedimentary rock or rotten rock. Soils in group of 47 have pH about 5.0-7.5. The soil series in this group are classified as Muak Lek, Li, Nakhon Sawan, Tha Li, Sop Prap, Phai Sali, Pong Nam Ron, Ngao, Khok Pru, and Hin Son. The characteristics of soil in group of 47 are shown in Table 2.6.

Table 2.6 The characteristic of soil in group of 47 at the depth of 0-30 cm

Soil series	**O.M. (%)**	**O.C. (%)**	**CEC (Meq/100g soil)**	**pH**	**Soil texture**	**Sand (%)**	**Silt (%)**	**Clay (%)**
Muak Lek	3.55	1.99	13.52	6.0-7.0	Clay loam	19.30	30.50	50.20
Li	4.50	3.81	24.43	6.0-7.0	Silt clay	9.20	50.00	40.80
Nakhon Sawan	0.95	0.63	14.60	5.5-6.5	Sandy loam	64.90	29.60	5.50
Tha Li	2.05	2.54	12.60	6.0-7.0	Loam	34.30	43.60	22.10
Sop Prap	3.76	2.81	61.47	6.5-7.0	Clay	24.00	23.00	53.00
Pong Nam Ron	6.28	3.06	32.13	7.0-8.0	Clay loam	-	-	-
Phai Sali	1.98	-	4.40	6.0-7.0	Sandy loam	-	-	-
Khok Pru	1.43	-	18.00	5.5-6.5	Loam	-	-	-
Hin Son	5.39	-	40.60	7.0-8.0	Clay loam	-	-	-

Source: Department of Land Development

2.6.3 Climate

(1) Climate of Nakhonratchasima Province

Nakhonratchasima Province is located on Tropical Savannah climate. It is very hot in summer and very cold in winter. The rainy season has begun in May to October and too much rain in August to October due to the influence of Southeast monsoon from the Indian Ocean. In 2006, the average annual rainfall was approximately 986.9 mm, 104 days of total rainfall. The highest rainy in October, was measured about 255.40 mm, 11 days of rainfall. The lowest rainy were measured about 0.0 mm in November.

In winter, it has begun since November to February due to the influence of the Northeast monsoon from cold wind of China. Summer has begun since February to May. The temperature was 18-29 degrees centigrade (°C), maximum in April (38 °C) and minimum in January (15.4°C).

The average annual relative humidity was approximately 72%. Maximum in September was about 82% and minimum in January was about 63%, (average monthly).

The average annual wind speed of Nakhonratchasima Province was about 1.9 km/h. Maximum in July was about 2.9 km/h and minimum in September was about 1.2 km/h, average monthly. The information derived from The Thailand Meteorological Department, 2006.

(2) Climate of the selected vineyard

The vineyard had collected the climatic information. In 2006, the average annual rainfall was about 1,543.30 mm, maximum in March (304.5 mm) and minimum in January and December (0 mm). The temperature was maximum in April about 36 °C and minimum in January and December about 11°C.

2.7 Standards/Guidelines for Mn and Cu

Many countries have standards or guidelines for environmental investigation, clean up and remediation. Foodstuffs are investigated, too. The standards or guidelines are shown below;

2.7.1 Soil quality standards/guideline

The standards were used to investigate the soil level of Mn and Cu from the study area which if it has high levels above the standard, it may be affected the biological environment and require the investigation or remediation. That is used to evaluate the environmental hazard from the Mn and Cu applications. The standards for Mn and Cu in soil were obtained from Pollution Control Department of Thailand, the Netherlands Ministry of Housing, Spatial Planning and Environment (Swartjes**,** 1999) and the Australia and New Zealand Environment and Conservation Council, National Health and Medical Research Council (1992), which are shown in Table 2.7.

Table 2.7 Soil Quality Standards for Mn and Cu

Metals	**Soil quality standards (mg/kg dw)**			
	[1]Thailand (Habitat and agriculture)	**[2]Netherlands (soil/sediment)**		**[3]Australia and New Zealand (requiring investigation)**
		Requiring investigation	**Requiring remediation**	
Cu	-	36	190	60
Mn	1,800	-	-	-

[1]Pollution Control Department, the Ministry of Natural Resources and Environment (2004)

[2] Swartjes (1999)

[3]Australia and New Zealand Environment and Conservation Council, National Health and Medical Research Council (1992)

dw = dry weight

The knowledge of background values of heavy metals in soil is necessary before a soil can be declared to be contaminated. The values can be defined as the natural concentration of heavy metals in soils without human influence (Salminen and Gregorauskiene, 2000). The Netherlands Ministry of Housing, Spatial Planning and Environment's Circular established national background concentration (BC) in soil/sediment, report showed value for Cu was 36 mg/kg dw. Carolina Micó (2006) investigated baseline values for Mn and Cu in soil of Alicante (Spain), the European Mediterranean regions. The baseline values were 402 mg/kg dw for Mn and 28 mg/kg dw for Cu.

Zarcinas (2004) proposed the 95th percentile value of the random selected agricultural and background soil sampled used as the minimum concentration of heavy metal for soil to be considered contaminated. The 'investigation levels' provide a threshold value, which can trigger further evaluation of possible contamination. The investigation level for Cu in soils of Thailand was 45 mg/kg dw. Soils with concentration above investigation concentration are likely to have been contaminated to significant extent due to addition of metals by fertilizers, wastes, pesticides and atmospheric sources. Moreover, government of Slovenia established limiting value of pollutants in soil in 'Directive of limited and critical values of dangerous substances in soil of Slovenia, Cu was 60 mg/kg.

As for sediment, quality guidelines were established in many countries as shown in Table 2.8.

Table 2.8 Sediment quality standards/guidelines

Metals	**Sediment quality guidelines for the protection of aquatic life (mg/kg dw)**			
	[1]Canada		**[2]Ontario**	
	Interim freshwater sediment quality guideline (ISQG)	**Probable effect level**	**Lowest effect level (LEL)**	**Severe effect level (SEL)**
Mn	-	-	460	1,100
Cu	35.7	17	16	110

[1] Canadian Council of Minister of Environment (CCME) (2001)

[2] Ministry of Environment and Energy of Ontario, 1993

2.7.2 Food standards/guidelines

Mn and Cu contain naturally in food but they are contaminated with high concentration when resulting from manufacturing process, method of manufacturing, site of manufacturing, storage, transportation and contamination from the environment. Mn and Cu are the essential elements for health but they can be toxic when expose exceed certain levels. There have the limit concentrations in food for Cu but for Mn are not certain. Table 2.9 showed Maximum permitted concentration (MPC) as state in the 'announcement of the Ministry of Health No.98, 1986, Standard of Contaminated Food, Ministry of Health, Thailand.

Table 2.9 Standards/guidelines for Cu contaminated in food

Standards/guidelines	Limit concentration (mg/kg food (ww))
1. Food contaminated toxic substance by the Ministry of Public Health, Thailand, 1988	20

2.8 Evaluation of the Potential Environmental Risks

Environmental risks assessment has occurred in the last thirty to forty years as the public has realized the adverse effects from human activities to the environment such as agriculture, industrial performances, automobiles emission, mining and waste disposals. The publication in 1962 of Rachel Carson: The Silent Spring, presented the harmful effects that pesticides have had on the environment, especially DDT. The 1970s saw the passage of environmental protection legislation such as the clean Air Act, the Clean Water Act and the Superfund environmental clean up regulation. All of these legislations require risk assessment.

U.S. EPA (1992) defined ecological risks assessment, is the process that evaluates the likelihood that adverse ecological effects may occur or are occurring as the result of exposure to one or more chemicals in the environment. An ecological risk assessment evaluates the potential adverse effects that human activities have on the environment (living and non-living environment) that make up ecosystem. The risk assessment process provides a way to develop, organize and present scientific information so that it is relevant to environmental decisions. The process can be used to identify vulnerable and value resources, prioritize data collection activity, and link human activities to their potential effects. Ecological risk assessment results provide a basis for comparing different management options, enabling decision-makers and the public to make better informed decision about the management of ecological resources.

The screening level risk calculation

Ecological risks can be estimated using the Hazard Quotient (HQ) approach. This is a ratio of the exposure estimate to the effect concentration considered to represent a safe environmental concentration or dose. This equation is obtained from US Environmental Protection Agency (1997). The HQ is calculated with the following equation 1 and 2:

1. HQ = EEC/Screening Benchmark
2. HQ = Dose/ Screening Benchmark

EEC = Estimated (maximum) environmental contaminant concentration at the site; how much contaminant is in the soil, sediment, or water (e.g. mg contaminant/kg soil)

Dose = Estimated amount of how much contaminant is taken in by plant or animal, in terms of the body weight of receptor (e.g. mg contaminant/kg body weight/day)

Screening Benchmark = Generally a No-Adverse Effects level concentration (NOAEL); if the contamination concentration is below this level, the contaminant is not likely to cause adverse effect

If:

HQ < 0.1, no hazard exists

HQ 0.1-1.0, hazard is low

HQ 1.1-10, hazard is moderate

HQ > 10, hazard is high (Lemly, 1996)

HQ values may range from less 0.1, with values less than 1.0 considered indicative of acceptable risks. If HQ is calculated to be equal or greater than one for a particular contaminant, that contaminant is referred to as a Contaminant (or Chemical) of Potential Ecological Concern (COPEC) or sometimes as a Contaminant of Interest (COI) or an Ecological Contaminant of Potential Concern (EcoCOPC). U.S. EPA has not recognized any official means of evaluating the size of the results of these calculations, only whether or not the HQ exceeds one which it is not relevant to a Screening Ecological Risk Assessment.

The repeated uses of pesticides and fertilizers can produce the contamination of Mn and Cu in soil environment at site. Commonly, Mn and Cu are the essential minerals for all organisms but it becomes toxic when its concentration is elevated high in environment. Hazard Quotient (HQ) is the important tool to evaluate potential environmental risk. It is simply the ratio of the contamination estimate to an effect concentration considered to represent a safe environmental concentration.

2.9 Related Researches

Abollino et al. (2002) investigated the distribution and speciation of ten heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti and Zn) in five agricultural soils of Piedmont Region (north-western Italy). Analytical determinations were performed by Atomic emission or atomic absorption spectroscopy after microwave sample dissolution in acid solution. Total metal concentrations fit in typical concentration ranges for unpolluted soils with the exception of Cd and Pb content in some horizons. Total metal contents in agricultural soils were range 360-1,260 mg/kg dw for Mn and 16-46 mg/kg dw for Cu. Typical literature ranges of these metals (mg/kg dw) were 20-10,000 for Mn and 2-250 for Cu. Common values were 1,000 mg/kg dw for Mn and 20-30 mg/kg dw for Cu.

Rodri′guez Marti′n et al. (2006) quantified the content of seven heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) and other parameters (the pH, OM, carbonates and granumetric fraction) in agricultural topsoil in Ebro, based on 624 samples collected according to an 8 by 8 km square mesh. The average concentrations (mg/kg dw) obtained were Cd (0.415±0.163), Cr (20.27±13.21), Cu (17.33±14.97), Ni (20.50±22.71), Pb (17.54±10.41), Zn (17.53±24.19), and Hg (35.6±42.05). The concentration levels are relatively low in areas of high pH and low organic matter content.

Micó et al. (2006) investigated baseline values for heavy metals in Alicante (Spain), a representative agricultural area of the European Mediterranean region, as a basis to identify and assess soil concentration processes at regional level. Fifty-four agricultural plots were samples and heavy metals concentration were analyzed for nine elements (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) by atomic absorption spectrometry (ASS) after acid digestion with HNO_3 and HCl in a microwave oven. Mean values of heavy metals analyzed in the study area were (mg/kg dw): 0.34±0.20 for Cd, 7.1±1.7 for Co, 26.5±5.9 for Cr, 22.5±8.9 for Cu, 13,608±3,107 for Fe, 295±61 for Mn, 20.9±5.1 for Ni, 22.8±16.1 for Pb, and 52.8±14.9 for Zn.

The baseline values were 0.7 mg/kg dw for Cd, 11 mg/kg dw for Co, 36 mg/kg dw for Cr, 28 mg/kg dw for Cu, 19,822 mg/kg dw for Fe, 402 mg/kg dw for Mn, 31 mg/kg dw for Ni, 28 mg/kg dw for Pb, and 83 mg/kg dw for Zn. The experience gained in this work further suggests that baseline values for heavy metals should be proposed in other areas. This is necessary to facilitate the identification of soil contamination processes over the whole European Mediterranean region as a basis to undertaking appropriate action to protect soil resource quality (Micó et al., 2007).

Huang et al. (2007) investigated concentration of Hg, Cd, Pb, Zn, Cu, As, Ni, and Cr in samples of agricultural soil, cereal, and vegetables from Yangzhong district, China. Compared to subsoils, the sampled topsoils are enriched in Hg, Cd, Cu, Pb, Zn, and As. Concentrations of Cr and Ni showed little spatial variation, and high Cu, Pb, and Zn contents correspond well to areas of urban development. In top soils, Pb, Cu, and Ni had arithmetical mean concentrations of around 30 mg/kg dw, with As recording around 10 mg/kg dw, Cd around 0.3 mg/kg dw, and Cr around 80 mg/kg dw. Hg has the lowest mean concentration (0.2 mg/kg), while the highest contents were recorded for Zn (98.1 mg/kg).

The contents of Cd, Hg, and total organic carbon (TOC) increase gradually to maximum values in the upper parts of soil profiles, while Cr and Ni occur in low concentrations within sampled profiles As, Pb, Cu, and Zn show patterns of slight enrichment within the surface layer. Compared to data obtained in 1990, Cd and Hg show increased concentrations in 2005; this is attributed to the long-term use of agrochemicals. Cr and Ni contents remained steady over this interval because they are derived from the weathering of parent material and subsequent pedogenesis. The measured As, Cu, Pb, and Zn contents show slight increases over time due to atmospheric deposition of material sourced from urban anthropogenic activity. Low concentrations of heavy metals are recorded in vegetables and cereals because the subalkaline environment of the soil limits their mobility. Although the heavy metal concentrations measured in this study do not pose a serious health risk, they do affect the quality of agricultural products (Huang, 2007).

Peris et al. (2007) analyzed heavy metal content (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in soils and the edible parts of two types of horticultural crops (leaf and inflorescence crops) from 30 agricultural fields in Castellón (Spain), a representative area of the European Mediterranean region. The contents of heavy metals in soils grouped by crop types (leaf and inflorescence) were 35.4 and 36.0 mg/kg dw for Cu, respectively and Mn were 408 and 379 mg/kg dw, respectively. Concentration of heavy metals (mg/kg dw) in edible parts of two horticultural crops (leaf and inflorescence crops) were 13.2 and 8.7, respectively for Cu and 63 and 21, respectively for Mn.

The analysis of crop heavy metal contents showed a higher absorption and/or accumulation of heavy metals in leaf crops than in inflorescence crops. Differences in crop characteristics seemed to be responsible for the differential accumulation of heavy metals. Furthermore, agronomic practices and other sources of heavy metals (atmospheric deposition) may also have some influence on crop accumulation. From these results, they concluded that it was not only highly necessary to reduce the consumption of agrochemicals within the region but also to carefully plan the location of industries in order to guarantee crop quality and avoid serious damage to human health (Peris et al., 2007).

Pietrzak and McPhail (2004) investigated total Cu concentration in some vineyard soils of Victoria, Australia, was up to 250 mg/kg compare to background levels of approximately 10 mg/kg. The Australian and New Zealand guidelines for the assessment of contaminate sites recommend that total Cu concentrations in soil exceeding 60 mg/kg required environmental investigation, and this level is exceeded in 8 of the 14 vineyards investigated. The focus of the research was to measure the accumulation, distribution and fractionation of Cu in contaminates and uncontaminated soils as a step towards understanding Cu existence in soils and its potential for availability to flora and fauna. Physical and chemical properties and total Cu concentration were measured in soil samples from four selected vine-producing regions of Victoria, including vineyard soils and adjacent background soils. Cu in surface soils (0-1 cm) and throughout soil profiles (maximum depth of 50 cm) was separated in following fractions: water soluble (WS), exchangeable (E), sorbed (S), easily reducible Mn (ERMn), bound to carbonates (CA), organically bound (OM), bound to Fe and Al oxide (FeOx), and residual (RES). The result showed that the conversion between copper fractions is slow, indicating that copper can stay active in soils for long periods of time, greater than tens of years, and may result in leaching and transport to deeper soil layer.

Rusjan et al. (2006) obtained an overview of transition metals, including Cd, Co, Cu, Mn, Mo, Pb, and Zn in soils of the sub-Mediterranean winegrowing region of Slovenia. Twenty-four soil sampling sites (22 vineyards, 2 forests) were selected to obtain a reliable set of data and possibly find differences in metal content. The sites differ in soil properties, vineyard age, landform, and soil depth. Soil depth and vineyard landform were found to have no significant influence on soil characteristics. Statistically significant differences in CEC (cmol/kg), pH, and loss on ignition were found between vineyard and forest soils. Vineyard soils with average metal content of Cd (1.4 mg/kg dw), Co (23 mg/kg dw), Cu (75 mg/kg dw), Mn (1251 mg/kg dw), Mo (1.7mg/kg dw), Pb (22 mg/kg dw), and Zn (101 mg/kg dw) can classified as less polluted, even though the content levels of Cd, Co, and Cu are higher than the limited values as defined by the Slovenian Directive. The contents of Cd and Co in forest soil were also higher than the limit values, which can only be explained by the parental material influence. The highest contents of Zn, Pb, Cu, and Mn were detected in topsoil layers, although the differences according to soil sampling depth were insignificant. The content of Cu significantly increases with a vineyard’s age. The study confirmed that main source of transition metal pollutant in the region is intensive viticulture practice, especially the use of Cu substances.

Mirlean et al. (2007) determined Cu concentrations of the vine-growing areas in Brazil from Cu-based fungicide application. The maximum value registered in this study was as much as 3,200 mg/kg dw, which is several times higher than reported for vineyard soils in temperate climates. Other pesticide derived metals accumulate in the topsoil layer, surpassing in the old vineyards the background value several times for Zn, Pb, Cr, and Cd. Cu is transported to deep soils’ horizons and can potentially contaminate groundwater. The soils from basaltic volcanic rocks reveal the highest values of Cu extracted with $CaCl_2$, demonstrating a high capacity of Cu transference into plants. When evaluating the risks of Cu's toxic effects in subtropics, the soils from rhyolitic volcanic rocks are more worrisome, as the Cu extracted with ammonium acetate 1 M surpasses the toxic threshold as much as 4-6 times

The concentration of metals contaminated in vineyard soil of this study were 36.5-3215.6 mg/kg dw for Cu, 11.5-369.4 mg/kg dw for Zn, 5.4-113.3 mg/kg dw for Cr, 2.4-66.7 mg/kg dw for Ni, 1.1-46.5 mg/kg dw for Pb, and 0.02-2.8 mg/kg dw for Cd (Mirlean, 2007).

Nasir et al. (2001) investigated trace metals residue in grape in Jordan, where grapes are mostly produced. Sixty-four grape samples from five territories. The collected samples were analyzed for Ni, Cu, Zn, and Pb. Heavy metal concentrations (mg/kg ww) of Cu at investigated site 1 to site 5 were 0.185, 0.213, 0.203, 0.201 and 0.109.

Cabrera-Vique et al. (2000) determined Mn in grape samples with difference twelve varieties by electrothermal atomization atomic absorption spectrometry from different region of France. The concentrations were range 0.139 to 1.490 μg/g as wet weight.

CHAPTER III
MATERIALS AND METHODS

Field survey was performed. Both soil and sediment samples were collected on December, 2006, to determine the concentration levels of Mn and Cu. Grape fruits were collected in ripen season of March, 2007. The Mn and Cu analysis was performed in laboratory of the Department of Sanitary Engineering, Faculty of Public Health, Mahidol University.

3.1 Study Area

The selected vineyard is located at Pak Chong District, Nakhonratchasima Province. The situation is on the edge of the Kao Yai National Park with 300-350 meters above sea level. The site has approximately 2,500 rais, of which 500 rais are used for vine growing with nineteenth year of operation (1 hectare = 6.25 rais). Because of a long time of operation, GAPs management, large size of plantation and large amount use of fungicides and fertilizers, this site was selected to investigate the environmental contaminations of Mn and Cu due to agrochemical application from viticultural activities. This can affect the environment directly and resulting in the contamination in food chain.

The selected vineyard is located on 14° 33′ degree North and 101° 14′ degree East, the south is close to a hill and downhill to the north. GPS, vineyard map and field survey were used to set sampling stations in this study. Location of this study is at approximate vertical grid reference of 741100-742300 and horizontal grid reference of 1610000-1612000. The location of selected vineyard was shown in Figure 3.1 and vineyard map presenting total area of vineyard was shown in Figure 3.2. From these Figure, areas of vineyard are divided into blocks.

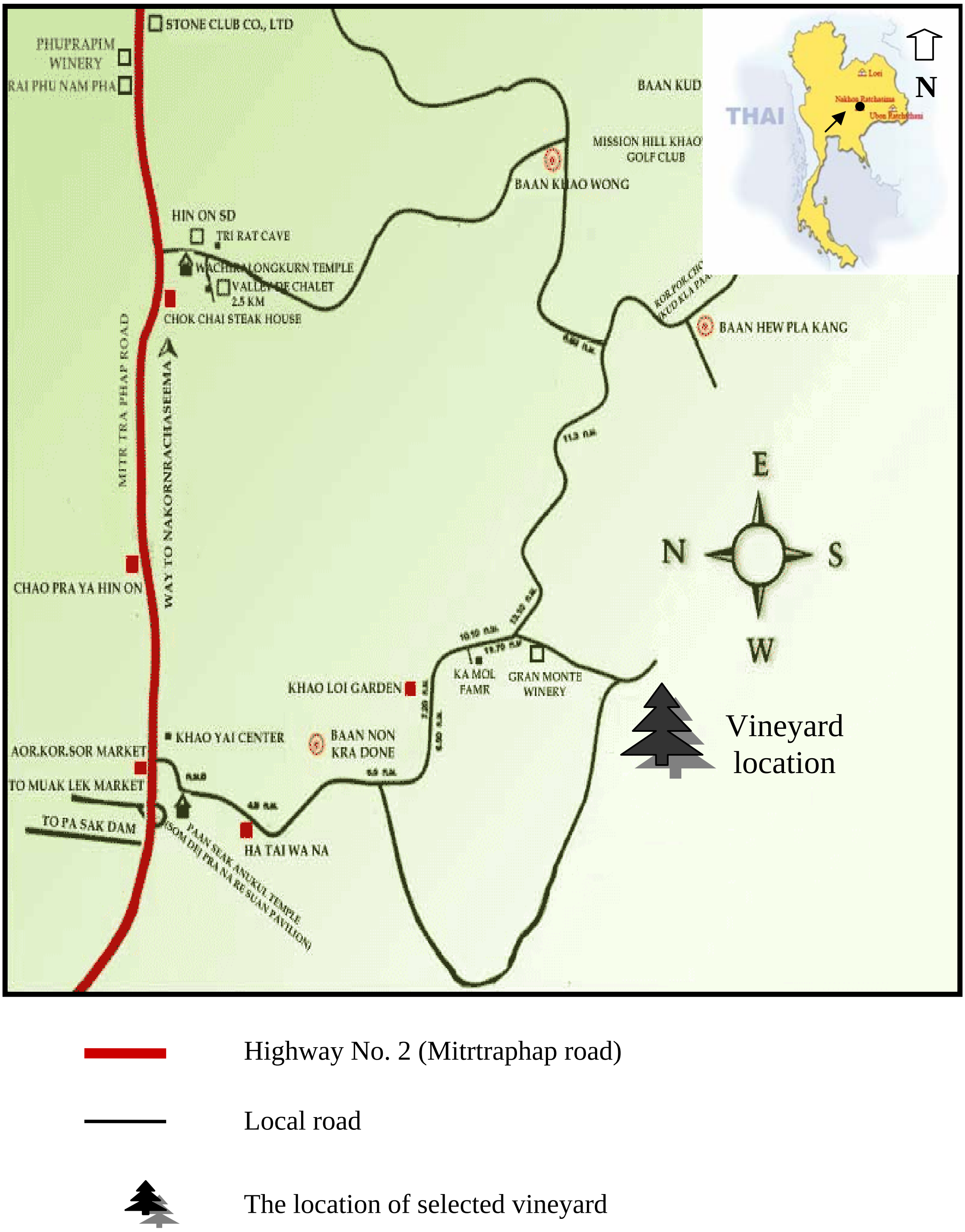


Figure 3.1 The map showing the location of selected vineyard

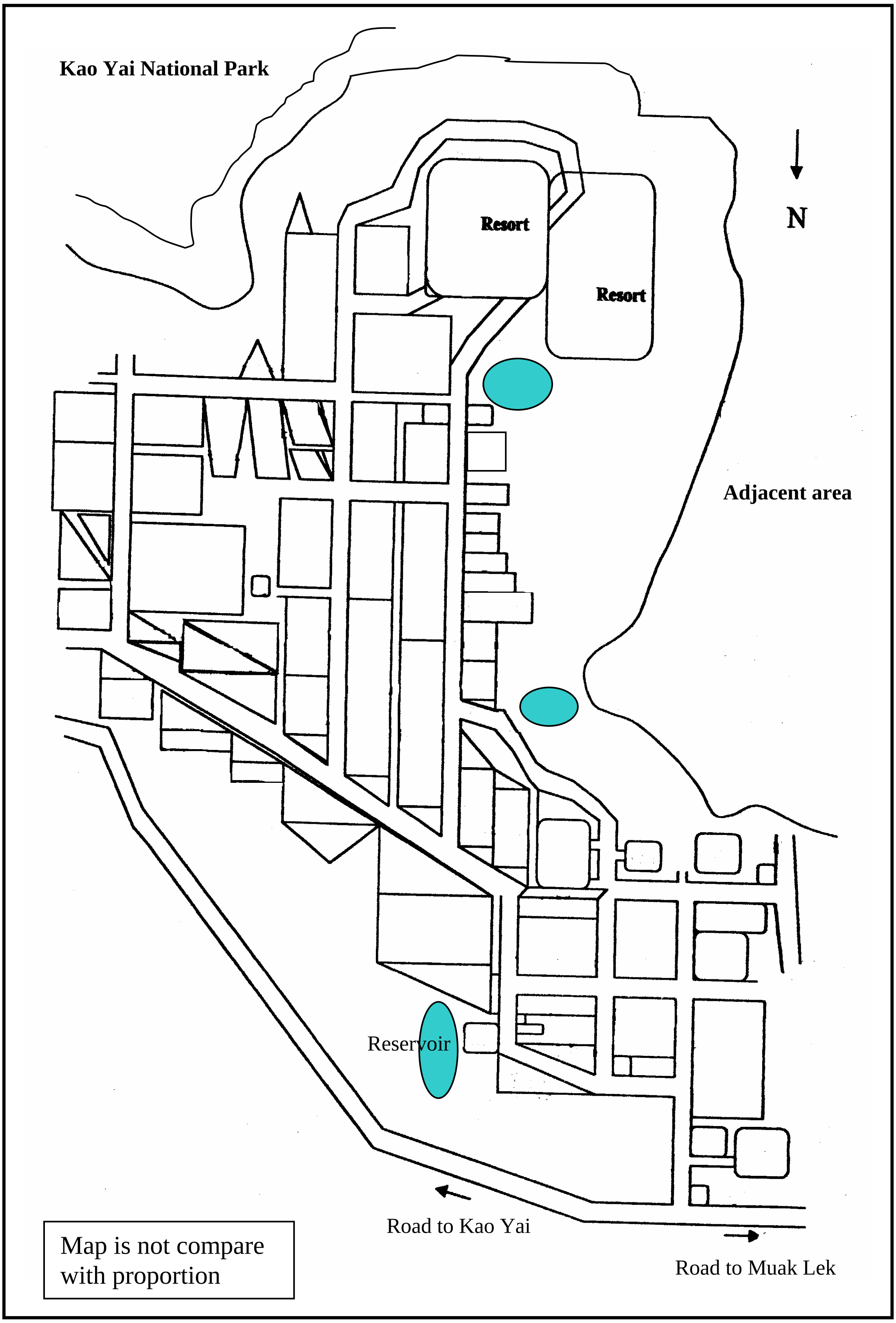


Figure 3.2 The map presented total area of vineyard (2,500 rais)

3.2 Sampling Designs

It was thought that heavy spraying of agrochemicals in vineyard introduces soil contaminations with heavy metals through both direct and indirect processes lead to contamination in sediments and plant tissues. In this study, metals were focused on Mn and Cu as components in these chemicals contaminated soils, sediments and grape fruits. The contaminations can affect human beings directly, through soil ingestion or through food web by crop ingestions, or indirectly, damaging environmental health (Peris, 2007). In order to set sampling designs, it was understood routs of contaminations and topographic feature of study area.

3.2.1 Sampling design for soils

The area of vineyard was divided into three transected lines in south-north direction. Samples were collected from uphill to downhill of vineyard in dry season (December, 2006).

In each transected line, the three sampling plots were randomly selected to collect the composite samples of surface soils (0-5 cm). Therefore, soils were collected at various stations/plots representing a total of nine samples.

Background or reference samples were collected in uncontaminated area of vineyard upwind away from the applications of pesticide and fertilizer with identical soil characteristics as those in vine plots. These sites are located on foothill in the south of vineyard. The two background soils were collected by composite sampling. Soil sampling stations was shown in Figure 3.3. R (R1 and R2) represented background or reference sampling station, other represented as contaminated soil sampling stations (set to S1-S9).

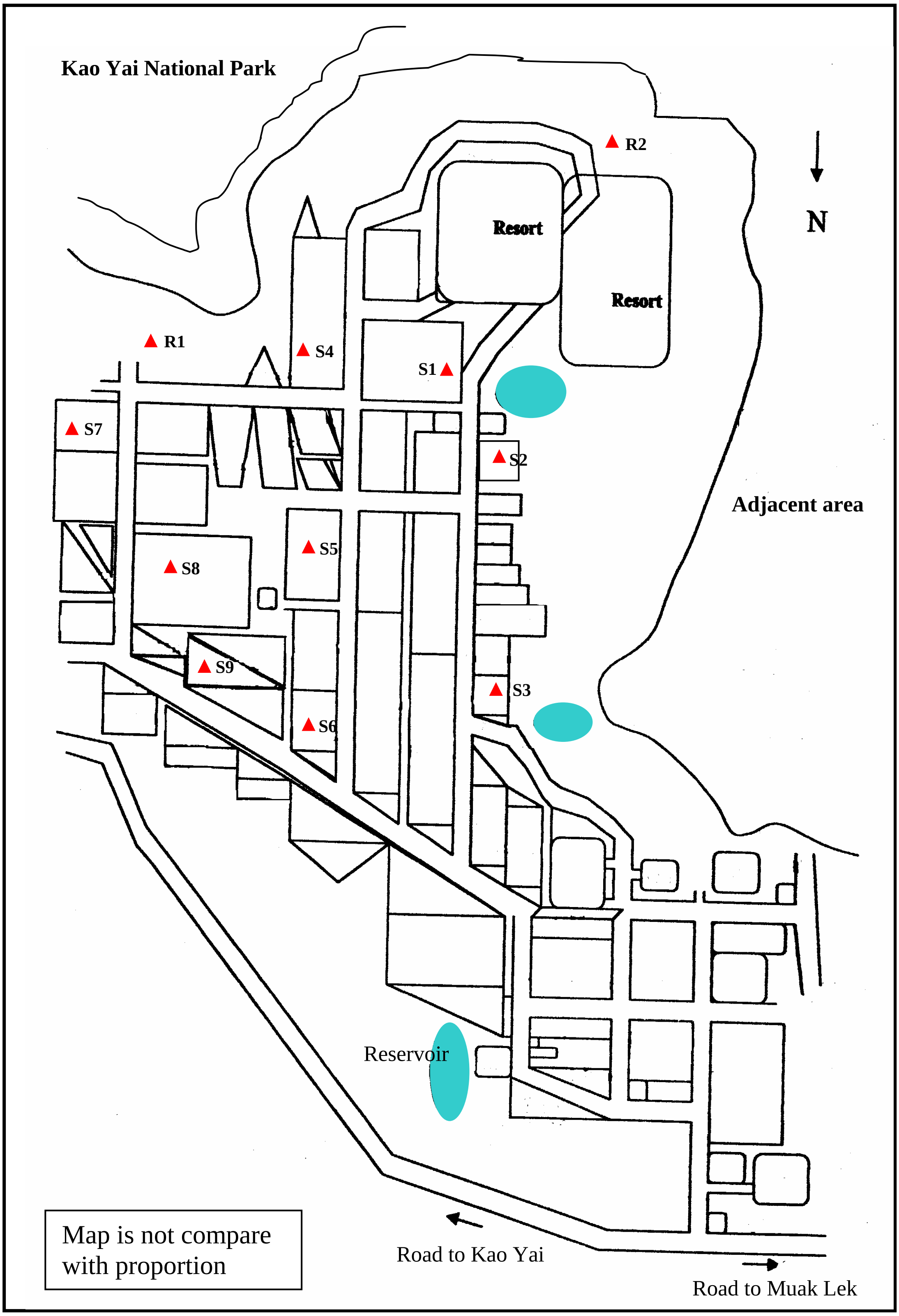


Figure 3.3 Soil sampling stations, S1-S9 contaminated sites, R1 and R2 background sites

3.2.2 Sampling design for sediments

According to divided vineyard into three transect lines. Sediment samples were collected from water drainage systems along vine sampling plots which the drainage systems perform as reservoir for collecting water from vine plot and beside that, sediments were collected at outlet points about 1 km downstream from the vineyard. Observation was performed on December, 2006. Twelve sediment samples were collected inside vineyard and the effluent discharges at public streams with distances of 50 m, 100 m, 500 m and 1,000 m from the effluents representative a total of eight samples. Sampling stations for sediment were shown in Figure 3.4. Set the sampling stations on SM1-SM20.

3.2.3 Sampling design for grape fruit

Ripen grapes were collected with composite sampling from vine plantation at the same location was done for surface soils on March, 2007, as shown in Figure 3.3. The nine samples were kept in ice box for transportation and kept in the freezer until analysis in laboratory. All grapes in this study were presented in *Vitis vinifera* species including table grape (Black Opal) and wine grape (Tempranillo, Chenin Blanc, Shiraz, Dornfelder and Colombard), as showed in Table 3.1.

Table 3.1 Grape sampling stations and their varieties

Soil sampling stations	Variety of grape	Type
S1	Tempranillo	Wine grape
S2	Black opal	Table grape
S3	Chenin blanc	Wine grape
S4	Black opal	Table grape
S5	Black opal	Table grape
S6	Shiraz	Wine grape
S7	Shiraz	Wine grape
S8	Dornfelder	Wine grape
S9	Colombard	Wine grape

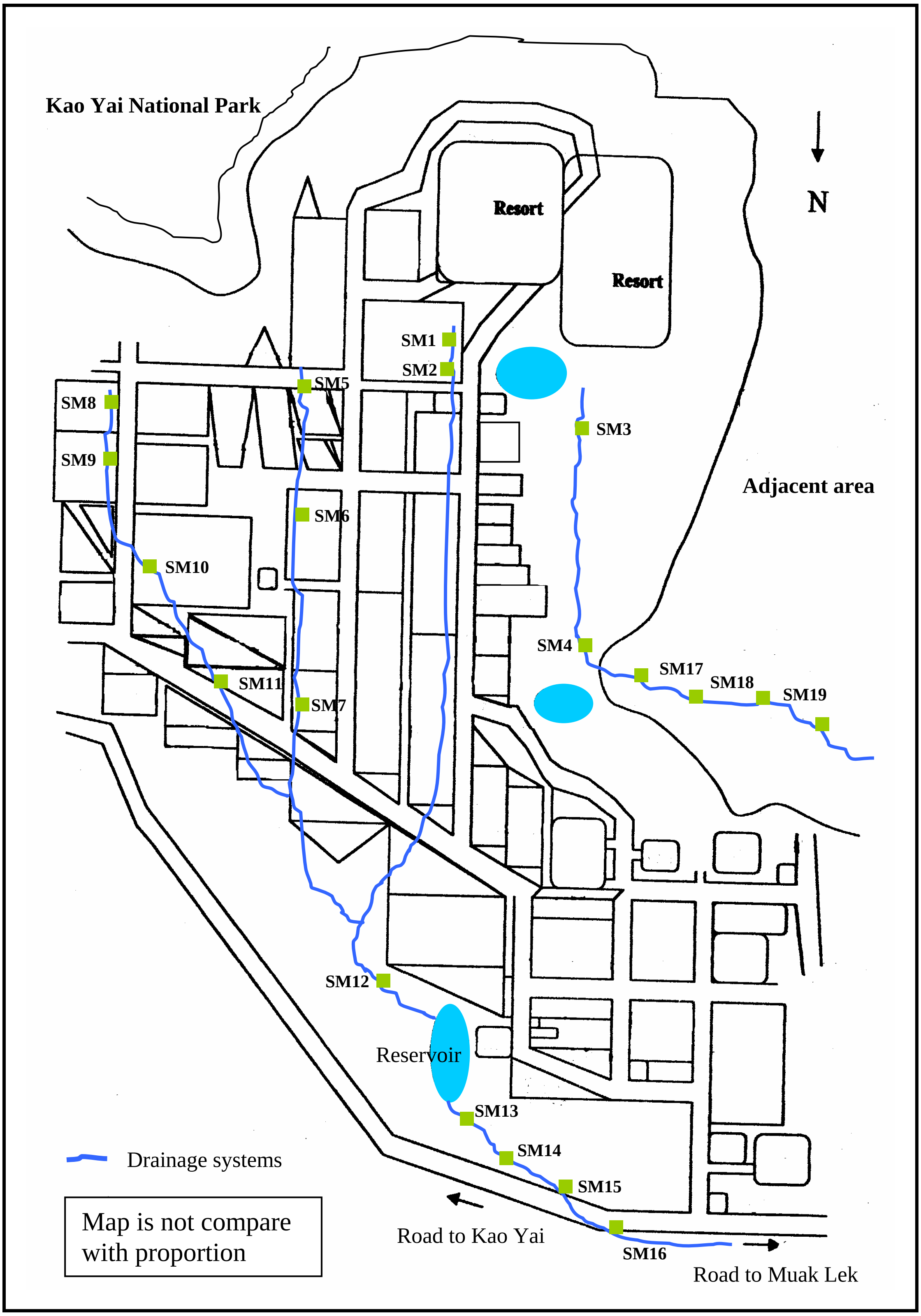


Figure 3.4 Sediment sampling stations, SM1-SM12 = Station inside vineyard, SM13-SM20 = Effluent discharge outside vineyard

Sampling designs for soil, sediment and grape were shown in Figure 3.5.

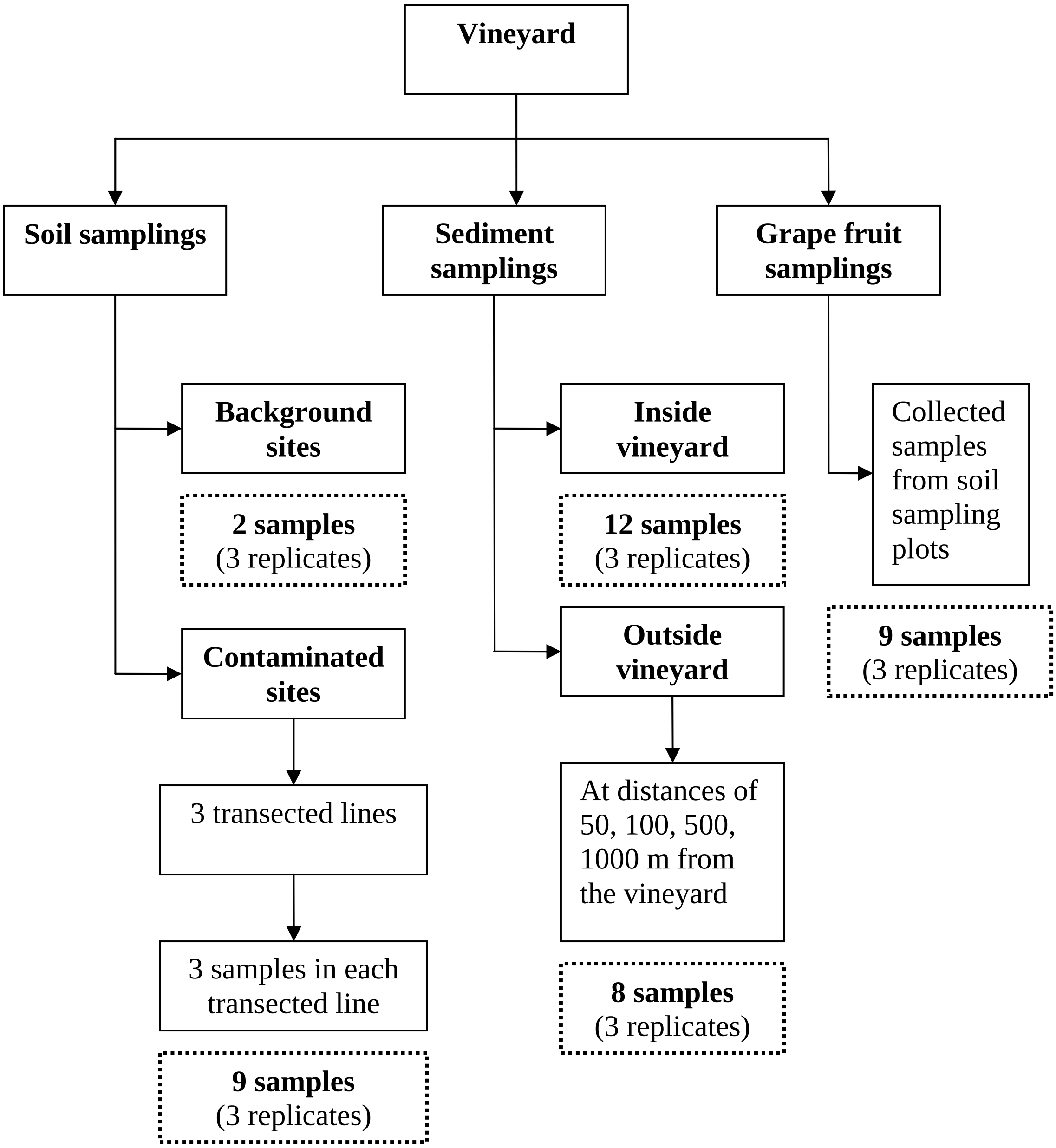


Figure 3.5 The sampling designs for composite sample of soils, sediments and grape Fruit

3.3 Sampling Methods

3.3.1 Equipment

1) Spade and spoon
2) Permanent pen and label
3) Zipper bags and plastic bags for sample collection
4) Ice box
5) Scissors
6) GPS and vineyard diagram

3.3.2 Sampling procedure

Labeled number of samples on zipper bag as use for sample container and noted the information about sampling station. The points of sampling station were recorded on vineyard diagram.

3.3.2.1 Soil sampling

1) Surface soils were collected using a spade with 5 cm of dept between vine rows in vine plot. The collection presented one vine plot was one sampling station.

2) In each vine plot, soils were collected randomly for four replicates. Each replicate was taken in 1 kg. All replicates were thoroughly homogenized at laboratory and then subsampled into two samples (each sample was approximate 1 kg).

3) Vegetable and debris were removed and discarded. The soil samples were taken in zipper bags and kept in the ambient air before analysis.

3.3.2.2 Sediment sampling

1) Sediments were collected from water drainage systems along vine plots in each transect line. Water drainage systems had done as reservoir and some were connected together. The drainage systems drain water off the vineyard. Then sediment samples were collected inside vineyard and collected at public stream outside vineyard at distance of 50 m, 100 m, 500 m and 1,000 m.

2) Sediments were scooped for 1 kg from the bottom of water drainage systems with 5 cm of dept and taken in zipper bags by digging with spade and spoon. Vegetable and debris were removed or discarded before kept.

3) All sediment samples were kept in ice box with temperature of 4 °C and sent to laboratory.

3.3.2.3 Grape sampling

1) Grape fruit was collected in the vine plot the same station as had done for soil with composite sampling (each vine plot, grapes were collected for four replicates around sampling plot). Samples were taken in zipper bags and kept in the ice box with 4 °C.

2) Samples were sent to laboratory and stored in freezer before analysis.

3.4 Sample Preparation

Soil and sediment samples were dried in ambient air for four days under shade and grinded with porcelain mortar and pestle. The samples were passed through a stainless sieve with 2 mm of wide to remove larger particles. Because of more than 90% of contaminates reported are expected to present in particles smaller than 2 mm (Dennis, 1995). All samples were kept in zipper bags in ambient air. Before acid digestion, samples were dried again in oven with 103 °C for 24 hours and kept in desiccator.

Grape fruit was analyzed in fresh weight. The fresh grapes were cleaned and free of extraneous substances including soil and dust particles, and foliar spray residues that may influence analytical result. Plant materials these were decayed and moldy should be discard. Grapes were washed in water and rinsed in deionized water. After decontamination, grapes were removed excess water and selected only the pulp of fruit (separated stalk and seed), cut in small pieces and took to digest (Plank, 1992).

3.5 Sample Analysis

3.5.1 Physical and chemical analysis of soil and sediment

The physical and chemical characteristics of soil and sediment were analyzed by the official of laboratory of Soil and Science at the Office of Science for Land Development, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. Parameters and method for analysis were shown in Table 3.2.

Table 3.2 Physical and chemical parameters and methods for analysis

Parameter	Unit	Analysis method	Reference
pH	-	Soil:Water 1:1 pH meter	Peech (1965)
Organic matter (OM)	%	Wakley & Black titration	Wakley and Black (1947)
Cation Exchange Capacity (CEC)	cmol/kg	Extraction with 1 N NH_4OAc pH 7.0	Chapman (1965)
Soil Texture	% (sand, silt, clay)	Pipette method	Reynolds (1993)

3.5.2 Metal digestion procedures for soil, sediment and grape

3.5.2.1 Instruments

1) Hot plate
2) Hot air oven (103 °C)
3) Beaker 50 and 100 ml
4.) Erlenmeyer flask 125 ml
5) Analytical balance
6) Pepettes10 ml
7) Filter paper: Whatman No. 42
8) Glass filter funnels

Glassware was cleaned with detergent, washed with tap water and soaked in 10% nitric acid overnight, rinsed with deionized water and dried in room temperature.

3.5.2.2 Reagents

1) Nitric acid (65% HNO_3)
2) Hydrochloric acid (37% HCl)
3) Deionized water

3.5.2.3 Soil and sediment digestion procedures

Metal content of soil and sediment were determined in aqua regia extracts for wet digestion of samples (Zarcinas, 2004). Procedures were:

1) Weighted 2 g of dry sample into 125 ml Erlenmeyer flask (Accurately weight). All samples were analyzed in three replicates of each sample.

2) Added 50 ml of deionized water into Erlenmeyer flask.

3) Added a mixture of Nitric acid and Hydrochloric acid with HNO_3: HCl in ratio of 1:3 (v/v), use Nitric acid 2.5 ml and Hydrochloric acid 7.5 ml. Covered beaker with glass funnel.

4) Samples were digested on hot plate and refluxed for 3 hours at 95 °C without boiling until the digests were clear with light color. After that digests were cooled in room temperature. The rim of Erlenmeyer flasks were rinsed with deionized water.

5) The digests were filtered with Whatman No. 42 filter papers and diluted into 50 ml of volumetric flasks with deionized water.

6) Mixed the solutions in volumetric flasks. The procedures for soil and sediment sample digestion were showed in Figure 3.6.

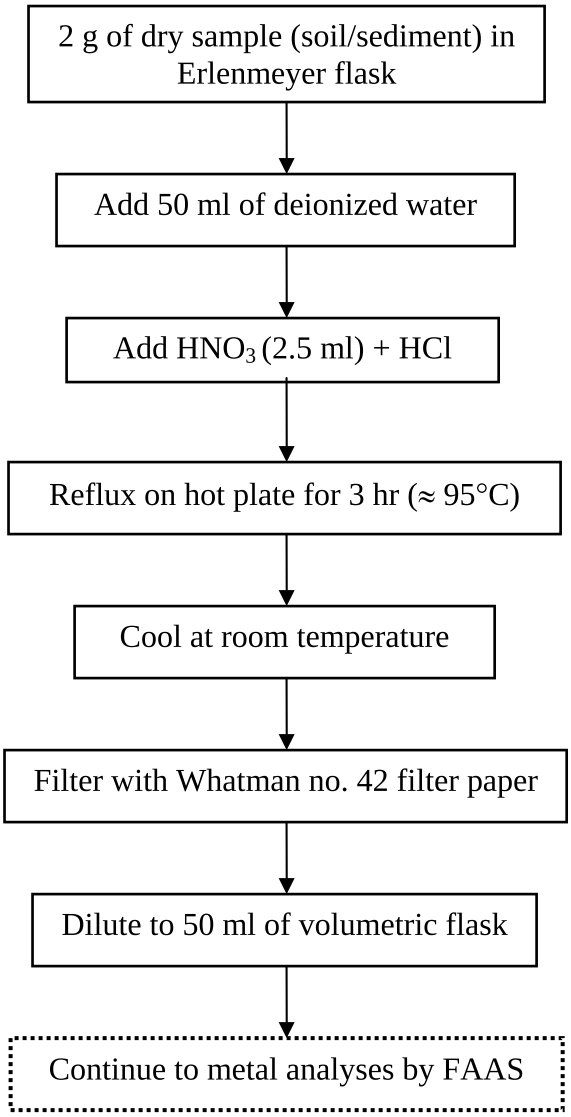


Figure 3.6 Soil and sediment digestion procedures (Aqua regia digestion)

3.5.2.4 Plant material digestion procedures

Metal content of grape fruits were determined using of Nitric acid wet digestion (Zarcinas, 2004). The procedures were:

1) Weighted 10 g of sample (fresh weight) into 125 ml Erlenmeyer flask (Accurately weight). All samples were analyzed in three replicates of each sample.

2) Added 50 ml of deionized water into Erlenmeyer flask.

3) Added Nitric acid 10 ml and cover beaker with glass funnel.

4) Samples were digested on hot plate and refluxed for 3 hours at 95 °C without boiling until the digests were clear with light color. After that digests were cooled in room temperature. The rim of Erlenmeyer flasks were rinsed with deionized water.

5) The digests were filtered with Whatman No. 42 filter papers and diluted into 50 ml of volumetric flasks with deionized water

6) Mixed the solutions in volumetric flasks. Procedures for plant material digestion were showed in Figure 3.7.

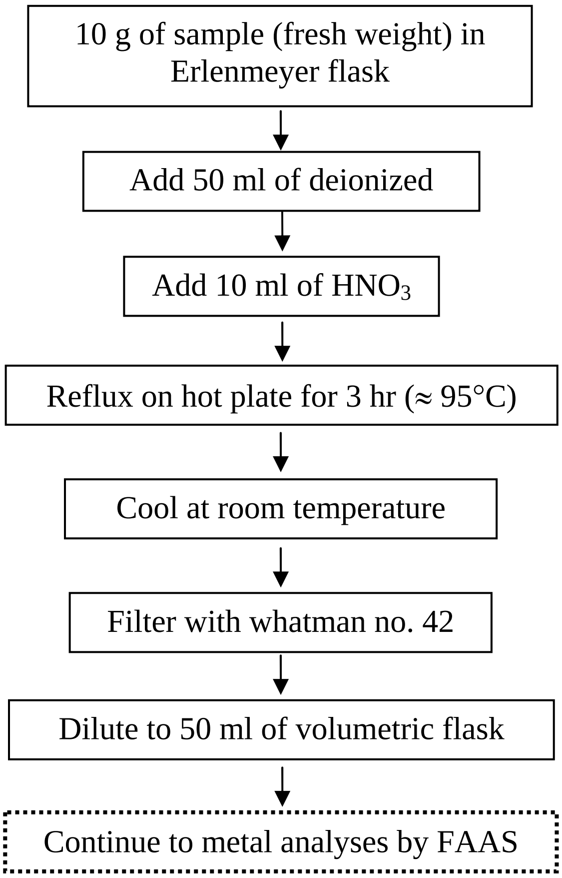


Figure 3.7 The procedures for plant material digestion (Nitric acid digestion)

3.5.3 Metals analysis

All digested samples (soil, sediment and grape fruit) were analyzed for Mn and Cu by Flame Atomic Absorption Spectrophotometer (FAAS). The operating parameters for Mn and Cu analysis showed in Table 3.3.

Table 3.3 Parameters for AAS Mn and Cu analysis

Metals	**Wavelength (nm)**	**Flame type**
Mn	279.5	Air- Acetylene
Cu	327.4	Air- Acetylene

3.5.4 The Quality control of laboratory

The accuracy of analytical procedure was calibrated using two replicated samples of standard reference materials, SRM 2709 (San Joaquin Soil: Baseline Trace Element Concentrations) for an agricultural soil and SRM 8704 (Buffalo River Sediment) for sediment from the National Institute of Standard and Technology (NIST), U.S. Department of Commerce and eight samples of blank. The percentage recovery of Mn and Cu were calculated compared with certified values.

$$\text{Recovery (\%)} = \frac{\text{Mean}_{\text{measured value}} \times 100\%}{\text{Mean}_{\text{certified value}}}$$

Method detection limit (MDL) for FAAS was calculated (by three multiplies the standard deviation of the eight reagent blank samples in the sample batch) and used as a tool for verification of all Mn and Cu analyses. The application of *t*-test for comparison showed that the certified and calculated values did not differ significantly (significance of 95%).

3.5.5 Metal Calculation

The total concentration of metals was calculated by this formulation and reported on dry weight or wet weight basis in mg/kg.

$$\text{Metal concentration (mg/kg)} = \frac{\text{Result from AAS (mg/L)} \times \text{Final volume of digest (ml)}}{\text{Weight of sample (g)}}$$

3.6 Data Analysis

Descriptive analyses were used to describe metal concentrations as mean, maximum, minimum and standard deviation.

Non parametric (Mann-Whitney U test) was used to test the difference between metal concentration in soil of contaminated site and background site ($p<0.05$). Correlation (Spearman) was used to define the relationship between metal concentrations and related factors included pH, CEC, OM, clay content and vine age (these statistical analyses were calculated using statistic software SPSS version 15.0).

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Site Characterization and Contamination

Viticulture is known to be the area of intensive uses of pesticides and fertilizers which a surplus of heavy metal in soil is caused by using these chemicals. A study on heavy metals contribution to soil through the applications of pesticides and fertilizers was thus performed on a vineyard located at Pak Chong District, Nakhonratchasima Province, Thailand. The vineyard has been operated for nineteen years more and managed in the manner of GAPs. In this manner, the operation is conducted with low persistent and low toxic substances recommended by the Ministry of Agriculture. Moreover, GAPs are designed for application of pesticides and fertilizers at appropriate moment and adequate dose. Therefore, there is no toxic substance such as persistent pesticides and high toxic heavy metal residues in this vineyard soil.

The study was found that Mn and Cu were the active components of pesticides which have been used for decades in this vineyard. Mn is an active component of mancozeb and Cu from a part of active ingredient of copper oxychloride. 20% of Mn-salt is found in a component of Mancozeb and 50% of Cu is contained in Copper oxychloride. Both of these chemicals are low toxic and low soil persistence but metals in their molecules are non biodegradable. They tend to be accumulated in soil as excessive use of these chemicals for long period of time (nineteen years).

Furthermore, fertilizer is known to be the important source of Mn and Cu contaminated in soil of the vineyard. Alloway reported that there were typical concentrations of Mn and Cu in fertilizer and farmyard manure. It was found that Mn and Cu in phosphate fertilizers were ranged from 40 to 2,000 mg/kg and from 1 to 300 mg/kg, respectively and in farmyard manure, Mn and Cu were ranged from 30 to 969 mg/kg and from 2 to 172 mg/kg, respectively (Alloway, 1995).

Mn and Cu added to soil by fertilizing and spraying has been assessed from the amount of agrochemicals commonly used in the vineyard. The applications of these chemicals are shown as follow;

- Copper oxychloride

This chemical is applied for table grape and wine grape 3-4 times/year (240 g/plot/year) and 4 times/year (12 kg/plot/year), respectively.

- Mancozeb

This chemical is applied for wine grape and table grape 8 times/year (12 kg/plot/year) and 3-4 times/year (concentration for application is not reported, however, expected concentration for wine grape is lower than that for table grape), respectively.

- Fertilizers,

Chemical fertilizers are applied for wine grape and table grape 12 times/year and 6 times/year (50 g/vine), respectively. Farmyard manures are applied 5 kg/vine 2 times/year for both wine grape and table grape.

As a result of GAPs management and agrochemical uses in this vineyard, quantitative monitoring of Mn and Cu in the vineyard soils is thus necessary to determine the scale and their pollution potential. Mn and Cu were investigated as pollutants of soil contamination and also consequential achieve effects on sediment and crop product (grape fruit). The concentrations of these metals were investigated in term of total metal content of surface soil, sediment and grape fruit as well as an accumulation in environmental media. To understand the behavior of Mn and Cu in soils, it is important not only to measure total concentration but also to study the distribution by using physical and chemical characteristics of soil and sediment.

4.1.1 The quality control of laboratory

The accuracy of the analytical procedure for Mn and Cu determination was calibrated against standard reference materials and found to be closed to the certified values as shown in Table 4.1 and Table 4.2.

Table 4.1 Analysis of standard reference of SRM 2709

Metal	Certified value (mg/kg dw)	Measured value (mg/kg dw)	Recovery (%)
Mn	538	496±21	92.2
Cu	34.6	32.7±0.9	94.6

Table 4.2 Analysis of standard reference of SRM 8704

Metal	Certified value (mg/kg dw)	Measured value (mg/kg dw)	Recovery (%)
Mn	544	513±2	94.2

4.1.2 Soil characterization and contamination

Soil sampling was performed once in dry season of December in the year 2006, as a representative of total contamination in this year. Samples were collected at surface soil based on period of viticultural operation (nineteen years) with 0-5 cm dept and analyzed on a basis of dry weight (dw). Soil sampling stations for Mn and Cu investigation were presented in Figure 4.1. R (R1 and R2) presented as background or reference sites which were taken up from uncultivated soils upwind away from fertilizing or spraying of pesticides. Background values were expected low or fit in common values/mean values as reported by Alloway (1990) as unpolluted soil. Stations S1 to S9 were presented as contaminated cultivated sites with different varieties of vine as shown in Table 3.1.

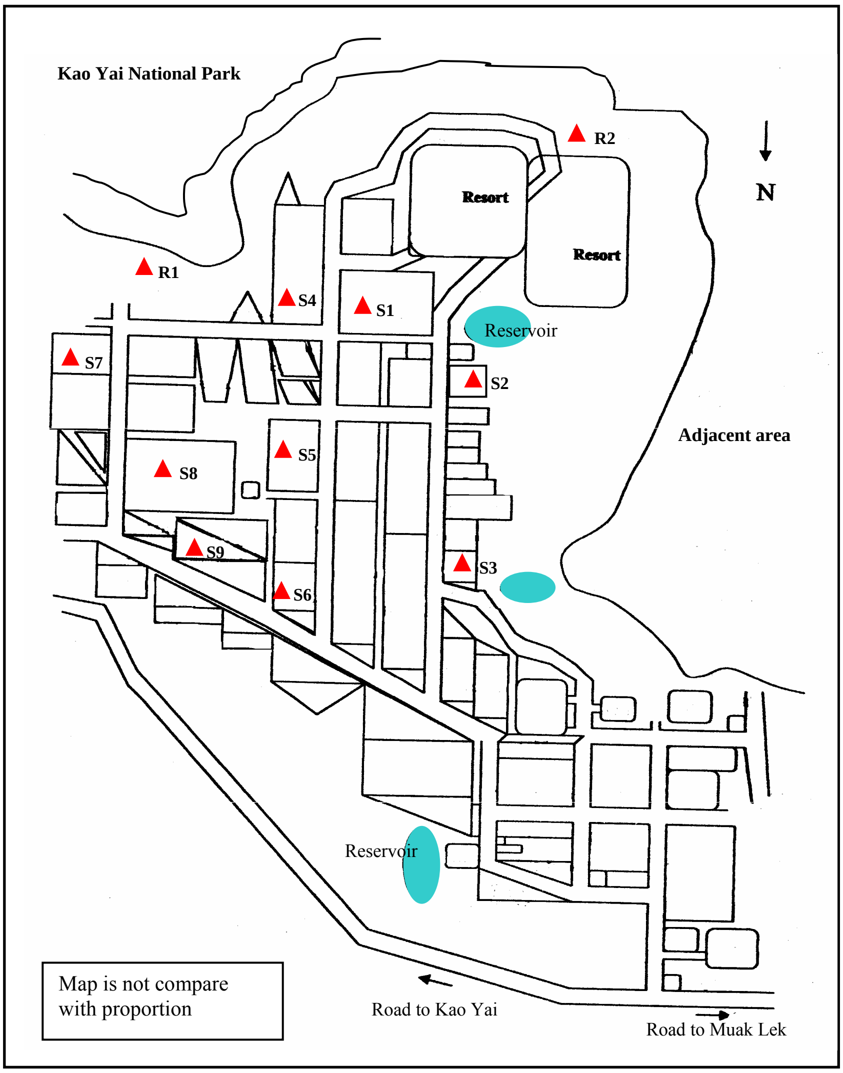


Figure 4.1 Soil sampling stations in vineyard (▲),

R = background sites

S = contaminated sites

Soil characteristics were determined and presented in Table 4.3. The results were found that most soils in study area had neutral pH (6.3-7.1), high organic matter (OM) (presented >2%) within the range of 3.89-7.55% and high cation exchange capacity (CEC) (14.56-22.83 cmol/kg). Particle size analysis showed that soils were clay loam textural class with 28.5-37% clay content. Such OM and clay texture have influence on soil mineral reserve. Soil with high contents of clay and OM strongly bind metals to its negative charged site and provides major reserve in top soil relatives to high value of CEC. The high value of CEC presents a large capacity of soil to adsorb cations and they are not easily lost when the soils are leached out by water. The soil properties exhibited low bioavailability of metal for plant.

Table 4.3 Physical and chemical characteristics of vineyard soil

Soil station	pH	OM (%)	CEC (cmol/kg)	Particle Size Analysis Sand (%)	Silt (%)	Clay (%)	Texture
R1	6.9	2.27	12.84	43.7	24.1	32.2	Clay loam
S1	6.9	3.89	18.77	30.2	34.8	35.0	Clay loam
S2	6.5	4.86	17.53	31.5	35.7	32.8	Clay loam
S3	6.5	4.89	16.68	33.9	37.6	28.5	Clay loam
S4	6.9	6.78	20.14	28.4	36.9	34.7	Clay loam
S5	7.0	4.49	20.96	25.4	37.6	37.0	Clay loam
S6	6.5	4.56	18.74	30.9	32.3	36.8	Clay loam
S7	7.1	7.02	16.26	29.4	34.5	36.1	Clay loam
S8	6.3	6.92	14.56	35.2	34.1	30.7	Clay loam
S9	7.1	7.55	22.83	28.3	35.4	36.3	Clay loam

In this vineyard, concentrations of Mn and Cu in soil varied in all sampling stations fit in the ranges of typical concentrations mentioned by Alloway (1990) as shown in Table 4.4. The elevated concentrations were found in soils of vine sampling plots at stations S1-S9 resulting from organic amendment and agrochemical applications in this site. These levels were significant greater than those of background stations ($p<0.05$). Mn concentration in soil was significant higher than Cu as a result of soil parent materials, contents of metals in agrochemicals (Mn showed higher content in many fertilizers than Cu as shown in Table 2.5) and the fact that mancozeb was sprayed with higher frequency than copper oxychloride. No correlation between Mn and Cu in soil of vine plots (correlation coefficient = 0.128) thus these metals were separately discussed.

In study area, Mn concentrations in soil at stations S1-S9 were ranged from 1,029 to 3,578 mg/kg dw compared with 571 to 600 mg/kg dw of soil from background stations (R1, R2). The soil standard for Mn according to PCD recommendation is 1,800 mg/kg. The results also showed that Mn values in soil of vineyard were not exceeded PCD’s standard (1,800 mg/kg dw) excepted in stations

S1, S2 and S4 which associated with the longer period of chemical application (age of vine).

Table 4.4 Mn and Cu concentrations (mg/kg dw) in vineyard soil

type of grape	**Vine age (year)**	**Sampling station**	[a] **Metal concentrations (mg/kg dw)**	
			Mn	**Cu**
Wine grape	10	S1	3,578	32
Table grape	19	S2	3,408	83
Wine grape	6	S3	1,163	46
Table grape	8	S4	2,640	34
Table grape	8	S5	1,243	37
Wine grape	10	S6	1,497	45
Wine grape	7	S7	1,294	28
Wine grape	5	S8	1,092	36
Wine grape	5	S9	1,029	31
		R1	600	16
		R2	571	21
		[b] Typical range	20-10,000	2-250
		[b] Common/mean	1,000	20-30

[a] Mean concentration (mg/kg dry weight)
[b] Typical range and common value mention by Alloway (1990)

Consideration of total Cu in soil, the results showed that the concentrations at stations S1-S9 were ranged from 28 to 83 mg/kg dw compared to those of two background stations (R1 and R2) which presented at low levels (16 and 21 mg/kg dw respectively). Thailand soil standard was not established limit concentration for Cu thus the Netherlands Quality Criteria for soil heavy metal contaminations was used in this study. This standard sets 36 mg/kg for target/background value and 190 mg/kg for intervention/polluted value. Site concentration less than target value indicates no restriction necessary and concentration between target and intervention value shows soil required further investigation or restriction may be warranted and exceed intervention soil remediation is necessary. The results exhibited Cu concentrations at the level that no potential toxic to soil environment was observed and no necessary remediation needed. However, vineyard soil is required further investigation for long term applications. Additionally, Slovenia's regulations established limit value of pollutants in soil according to 'Directive of limited and critical values of heavy metals in soil of Slovenia' (Directive, 1996) of Cu was of 60 mg/kg dw. In Australia and New Zealand, Cu concentration exceeding 60 mg/kg dw in soil requires environmental investigation (Australian and New Zealand Environmental and Conservation Council, National Health and Medical Research Council, 1992). Fortunately, in this vineyard, Cu contents were mostly found to be less than these two standards accepted in station S2 which presented up to 83 mg/kg dw related to vine age and cultivated period.

The statistical analysis exhibited positive correlation between Mn and Cu at various stations and vine age. It also revealed that metal concentration of soil increased as increasing period of exposure or cultivation. It indicated that these two elements could exist in the vineyard soil and there was elevated concentration when it has long period of cultivation (in term of vine age). In particular cases, there were difficult to establish a link between vine age and metal contents because it has many factors affecting metal concentration in this soil. Variation in metal contents for all sampling stations could be then explained by this way. The metal accumulation in soil was observed without knowing the actual periods of application of metal containing agrochemical.

The accumulation also depended on the rate of applications (In fact, a wine grape was sprayed with higher concentration and more frequency than a table grape) of agrochemicals (in both concentration and composition of metals). The results also showed that there was no relationship between metal concentration in soil and rate of application for wine grape and table grape. The concentrations of metal at station SM 4 (table grape) were significant higher than that of station SM 6 (wine grape) that indicated no relationship between metal concentration and rate of application.

In fact that pesticides and fertilizers could have been applied for decades, there was not possible to ascertain the history of the vineyard and actual period of cultivation whether it had another agricultural practices on this area before viticulture. The result showed that metal concentration in soil of the vine plot at the same vine age and type of vine was significant different (S1 compared to S6). Due to there were other factors affecting the metal concentration.

By comparison with the other study of Rusjan, Strlič, Pucko, Šelih and Korošec-Koruza in the year 2006 which reported that heavy metal contents in soil of winegrowing region of Slovenia were in the range of 1,174 to 1,368 mg/kg dw and 65 to 87 mg/kg dw for Mn and Cu respectively, with 5-20 years of exposure (in term of vineyard age).

In the year 2007, Mirlean, Roisenberg and Chies reported that Cu concentrations in soil of vine growing area in Brazil were ranged from 36.5 to 65.3 mg/kg dw with twenty years of operation (Mirlean et al., 2007).

Likewise, Pietrzak and McPhail (2004) reported that Cu concentrations in soil of winegrowing region, Goulburn Valley, in Victoria, Australia were ranged from 63.4 to 76.7 mg/kg dw. The Cu level was conducted in vineyards with the 20-30 years of operation period.

Table 4.5 Average concentrations of metal in vineyard soil

Metal	**Mean concentration (mg/kg dw)**			
	This study (Thailand)	**Mirlean et al. (2007) (Brazil)**	**Rusjan et al. (2006) (Slovenia)**	**Pietrzak and McPhail (2004) (Australia)**
Mn	1,883	-	1,368	-
Cu	41.3	50.1	87	68.5

Although this vineyard was operated with GAPs management, the results still showed the soil contamination with Mn and Cu. It revealed that the average Mn concentration in this vineyard soil was still elevated high above Thailand soil quality standard (1,800 mg/kg) and also higher than that reported by Rusjan in the year 2006. As for Cu, the average concentration in the vineyard exhibited lower concentration compared to another study in the same period of cultivation (Table 4.5). In fact, this vineyard was found to be applied with mancozeb at higher frequency than copper oxychloride. Whereas in the other studies, the vineyard were found to be preferably applied with Cu-fungicide.

Overall of presented vineyard soil was not extremely toxic to the environment. The remediation was not necessary because soil properties of adsorption and soil pH provided metals to be non bioavailable form (less toxic) and their toxicity of these pollutants exhibited low. However, they can affect or have an adverse effect which toxic to microorganisms or often soil biota and can be then have an influence on soil fertility at this site. Additionally, the Mn and Cu concentrations would be increased with longer period of cultivation and could be then accumulated and strongly fixed in vineyard soil as an influence of OM, clay content and % CEC of this site as well. Thus the cultivation of vine is required further environmental investigation for soil pollution from viticultural practices.

Crop contamination

Soil contamination of heavy metal can affect human being indirectly via plant uptake by root through food web and then accumulated in production. In this study, the investigation was performed on an edible part of study plant (grape fruit) which relevant concerns in the dietary. As a result of soil contamination, bioaccumulation of Mn and Cu were investigated in grape fruit at the same sampling stations as was done for soil. Samples were collected once at ripen season of March, 2007, which were analyzed and reported on a wet weight (ww) basis as a form to be consumed. No correlation between Mn and Cu concentration in grape fruit and vineyard soil was found and the results also showed the significant different between mean content of Mn and Cu. The Mn contents in grape fruit were ranged from 0.79 to 3.82 mg/kg ww and as for Cu, the concentrations were ranged from 0.39 to 1.82 mg/kg ww (Table 4.6).

The maximum permitted concentration as stated in the announcement of the Ministry of Health (Thailand) No. 98, 1986, standard of contaminated food, presented level for Cu at the concentration of 20 mg/kg ww. The results showed that Cu contents of grape fruit were not exceeded this standard with average concentrations about 0.73 mg/kg ww. There were no permitted concentration or standard for Mn in food presented but the concentration in this study presented at low level with the average of 1.64 mg/kg ww.

Table 4.6 Metal concentrations in grape fruit at ripen season of March (2007)

Station	Type of grape	Variety	Concentration (mg/kg ww)	
			Mn	Cu
S1	Wine grape	Tempranillo	2.31	0.65
S2	Table grape	Black opal	1.61	1.13
S3	Wine grape	Chenin blanc	0.87	1.82
S4	Table grape	Black opal	1.10	0.44
S5	Table grape	Black opal	1.33	0.39
S6	Wine grape	Shiraz	2.15	0.80
S7	Wine grape	Shiraz	0.80	0.48
S8	Wine grape	Dornfelder	3.82	0.49
S9	Wine grape	Colombard	0.79	0.40
Average			1.64	0.73
Food standard			-	20

There is no significant correlation between total Mn in soil and Mn content in grape fruit whereas grape fruit content has significant negative correlations with pH and OM. In addition, increasing pH and OM resulted in decreasing in metal concentration of grape fruit. On the other hand, Mn in grape fruit has no correlation with CEC and clay content (but correlation coefficient presented negative way). As for Cu, the concentration of heavy metal in vineyard soil had positive correlation with its content in grape fruit. Statistical analysis showed that Cu in grape fruit had negative correlation with soil pH, CEC and clay content that exhibited metal content decreasing as increasing of soil pH, CEC and clay content. But there was no correlation between Cu content in grape fruit and OM (but correlation coefficient showed negative direction).

The result also exhibited low concentrations of Mn and Cu in grape fruit for all sampling stations which could be explained through the adsorption capability of soil (Table 4.3) including pH, CEC, organic content, soil composition (% sand and clay) and other important factors. Total heavy metal concentrations in vineyard soil are generally less important to vine than the bioavailability. Brun (2001) gave a definition for bioavailability which is a portion of soil metal that is available for intake into given organisms. Mn and Cu are available for plant adsorption in divalent state as Mn^{2+} and Cu^{2+}. The effect of soil pH is on the solubility or availability of minerals directly. Before metals can be uptaken by plants, it must be dissolved in soil solution. Most metal are more soluble or available in acid soil than in neutral or slightly alkaline soil. The availability of Mn and Cu for plant uptake is at pH below 5.0 resulting in excessive availability and thus toxic to plant. The tendency of metal soluble or available adsorbs to soils still depends on CEC and the organic composition of soil (ATSDR, 2000). A large CEC exhibits a large capacity to adsorb cations and soil component which varied in ability to attract and hold ions. Gupta and Aten (1993) reported that the bioavailability of metal has also decreased as increasing CEC and organic content. OM and clay mineral have high capable for hold ion to much larger degree compared with sand and then metals are not easily lost (Pennisi and Thomas, 2005). The results also revealed that high value of CEC and OM which presented capability for metal retention in this compartment. Moreover, neutral pH resulted in less available of Mn and Cu in soil and could be then explained for low concentration in grape fruit samples.

Differences in soil properties indicated the difference of Mn and Cu concentrations in grape fruit samples. Besides, difference of metal concentrations in grape fruit still depended on species or varieties of plant. Vine varieties were different in ability to absorb and accumulate different types of metals. Such difference could occur between different parts of vine due to a metabolic process for metal transportation and bioaccumulation. As a result, Mn and Cu might be accumulated with high concentration in other parts of vine than in grape fruit.

Statistical analysis showed that the concentrations of Mn in grape fruit were significant higher than those of Cu ($p<0.05$). It could be explained through specific characteristic of the different properties between these metals. It has been reported that Cu is strongly bound to organic material and clay mineral. Exchangeable Cu is held much more tightly than the other cations, and is not readily available to plant. Due to the neutral pH of soil, Cu leached very little allowing the heavy metals accumulated in soil. Additionally, the distribution of Cu in soil solution is less affected by changes in pH with in the range of normal pH encountered in environment (ATSDR, 2004).

The results also revealed that the bioaccumulations of Mn and Cu in grape fruit of this vineyard were rather high compared with the other studies (Table 4.7). In the year 2000, Cabrera-Vique, Teissedre, Cabanis, and Cabanis reported that Mn accumulations in grape fruit of different twelve varieties in France were ranged from 0.139 to 1.49 mg/kg ww (Cabrera-Vique et al., 2000).

In the year 2001, Nasir, Jiries, Batarseh, and Beese reported that Cu concentrations in grape fruit from Jordan were ranged from 0.185 to 0.213 mg/kg ww (Nasir et al., 2001).

Table 4.7 Concentration range of heavy metal in grape fruit

Metal	**Range of concentration (mg/kg ww)**		
	This study (Thailand)	**Cabrera-Vique et al.(2000) (France)**	**Nasir et al. (2001) (Jordan)**
Mn	0.79-3.82	0.139-1.49	-
Cu	0.39-1.82	-	0.185-0.213

4.1.3 Sediment characterization and contamination

Mn and Cu in sediments were investigated to identify the distribution of these pollutants from the vineyard to public aquatic environment. Observation was performed along water drainage systems of vine sampling plots inside vineyard and effluent discharge points at the distances of 50 m, 100 m, 500 m and 1,000 m from the discharge point of the vineyard in dry season the same time as was done for soil. Sampling stations for sediment were shown in Figure 4.2, station SM1 to SM12 were located along the drainage systems inside vineyard whereas station SM13 to SM20 exhibited the locations at various distances away from the effluent discharge points of reservoir. Characteristics of sediment were shown in Table 4.8.

Table 4.8 Sediment characteristics at various stations

Station	pH	OM (%)	CEC (cmol/kg)	Particle Size Analysis			
				Sand (%)	Silt (%)	Clay (%)	Texture
SM3	6.9	7.09	20.74	19.8	45.4	34.8	Silty clay loam
SM4	7.6	3.72	20.74	37.7	31.5	30.8	Clay loam
SM5	7.1	4.95	27.87	12.8	43.3	43.9	Silt clay
SM6	7.4	3.26	17.23	43.2	33.4	23.4	Loam
SM7	7.8	3.57	20.08	30.0	40.0	30.0	Clay loam
SM8	7.4	3.42	18.33	34.8	33.8	31.4	Clay loam
SM10	7.1	2.65	16.35	18.0	49.4	32.6	Silty clay loam
SM12	7.4	4.18	19.86	15.8	49.3	34.9	Silty clay loam
SM13 (50 m)	7.1	2.19	14.71	31.2	40.7	28.1	Clay loam
SM14 (100 m)	7.3	3.26	19.97	20.3	41.4	38.3	Clay loam
SM15 (500 m)	7.4	2.96	18.88	25.3	40.8	33.9	Clay loam
SM16 (1,000 m)	7.5	1.73	11.08	58.0	24.8	17.2	Sandy loam
SM18 (100 m)	7.8	4.18	19.97	31.5	41.0	27.5	Clay loam
SM19 (500 m)	7.6	5.25	24.47	33.4	45.2	21.4	Loam
SM20 (1,000 m)	7.4	3.72	14.16	62.9	23.6	13.5	Sandy loam

SM3, SM 4, SM 5, SM6, SM7, SM8, SM10 and SM12: Sediment stations in vineyard
SM13-SM20: Effluence discharge points from reservoir

Sediments of vineyard showed neutral pH with the range of 6.9 to 7.8, high level of CEC from 16.35 to 27.87 and more than 2% of OM. Clay contents varied from 23.4 to 43.9 that characterized from coarse texture to fine texture. Based on contents of clay and CEC, sediments in the study area showed strong capability for retention or binding of metals in their compartments which prevented metal from leaching or lost. Moreover, such pH showed the characteristic of low solubility of metal.

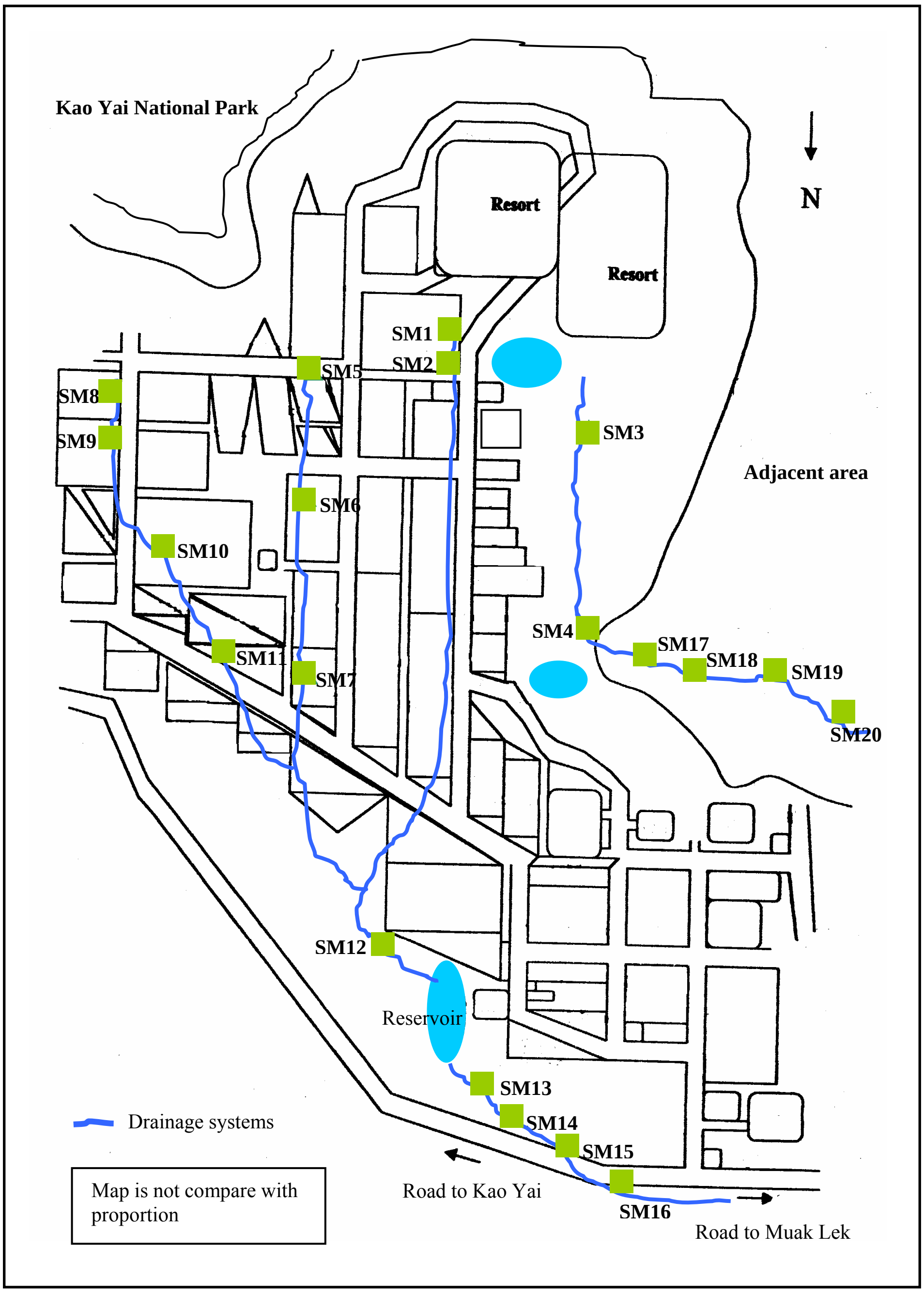


Figure 4.2 Sediment sampling stations (🟩), SM1-SM12 = Station in vineyard, SM13-SM20 = Station of effluent discharge points at public stream

In fact, the drainage systems of the vineyard perform as reservoir for collecting water in vine plots in rainy season (water runs from the South to the North) which then the heavy metals in surface soil and fertilizer in vine plot can be leached out to be metal containing particulate matters (as be influenced by weathering process and geographical feature of place which present slightly slope). These contaminated particulate matters were transported and settled out to the bottom of the aquatic tracks resulting in Mn and Cu contaminations. After that, they were discharged into small public streams. The samples collected from the stations at the distances of 50 m, 100 m, 500 m, and 1,000 m from the reservoir discharge point in two directions (north and west of the site) were investigated for distribution evaluation. Mn and Cu concentrations in sediment were determined on a basis dry weight (dw) at 0-5 cm of dept as shown in Table 4.9.

As for Mn, the contents in contaminated sediment (stations SM1 to SM12) were found to be in the range of 526 to 4,490 mg/kg dw. At the station SM 16 and SM20 located at far away distance sites about 1 km from contaminated area Mn contaminations presented the concentration of 529 and 761 mg/kg dw respectively, which could be then performed as background sites. Elevated concentrations in vineyard were found at stations SM1, SM2, SM4 and SM6 with Mn levels above 3,000 mg/kg dw. Sediments in these stations were collected from the inlet of drainage systems which directly drained water from vine plots. Therefore these stations might be the sinks of heavy metal and might be immobilized as a result of sediment characteristics and slow water flow of drainage systems by aquatic weed.

Consideration of Cu, most contents in the vineyard exhibited low concentrations with the range of 21 to 36 mg/kg dw when compared with those of stations SM16 and SM20 at far distance sites about 1 km from vineyard which exhibited the concentration of 12 and 18 mg/kg dw, respectively. Thus, the latter could be then represented as background concentrations. Exception in station SM3 and SM5, which were the dumping sites of agrochemical where agricultural instruments were cleaned, analyses showed high concentrations with 156 and 132 mg/kg, respectively.

Table 4.9 Mn and Cu contents of sediments at various stations

Station	**Description**	**Concentration (mg/kg dw)**	
		Mn	Cu
SM1	Upstream	3,170	25
SM2	Upstream	3,237	23
SM3	Upstream	1,687	156
SM4	Upstream	3,582	33
SM5	Upstream	1,668	132
SM6	Upstream	4,490	36
SM7	Upstream	1,663	23
SM8	Upstream	1,825	27
SM9	Upstream	1,247	25
SM10	Upstream	1,536	21
SM11	Upstream	526	23
SM12	Upstream	1,255	21
SM13	Downstream (50 m)	759	17
SM14	Downstream (100 m)	894	24
SM15	Downstream (500 m)	534	21
SM16	Downstream (1,000 m)	529	12
SM17	Downstream (50 m)	3,052	21
SM18	Downstream (100 m)	3,057	23
SM19	Downstream (500 m)	2,604	21
SM20	Downstream (1,000 m)	761	18
Standard	[1]Canada (2001)	-	35.7
	[2]Ontario (1993)	1,100	110

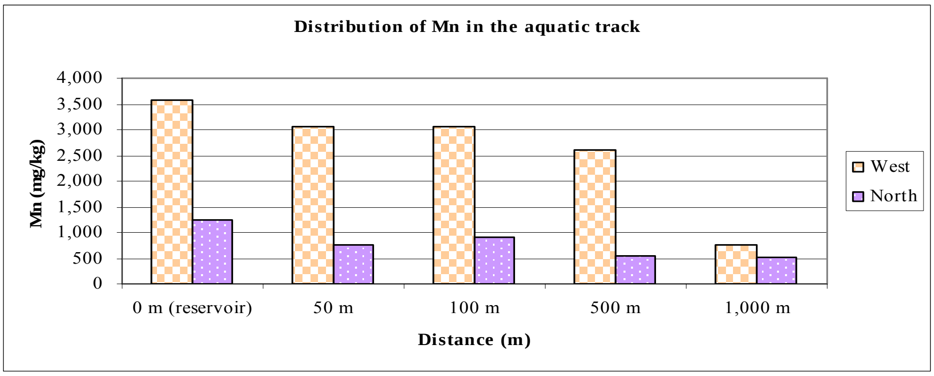
[1] Canadian Council of Minister of Environment (CCME) (2001): Interim freshwater sediment quality guidelines for the protection of aquatic life

[2] Ministry of Environment and Energy of Ontario (1993): Severe effect level

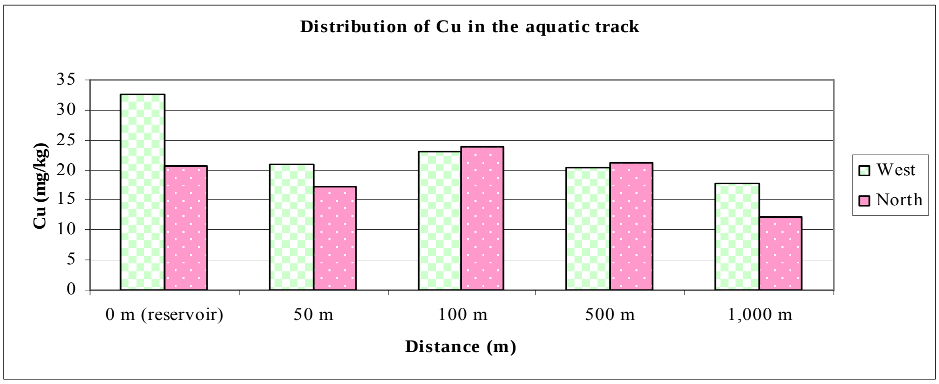
Thus the concentrations of Mn contaminated in sediment of vineyard were higher than those of Cu in all sampling stations. With respects of the soil characteristics of parent materials, metal contents in fertilizer, the strongly bound complex with clay as well as organic content, Cu is difficult to be leached out from soil.

In this study, Cu standard for sediment established by the Canadian interim fresh water sediment quality criteria was used as well as the Ontario sediment quality criteria. It was found that Mn contents of sediment in the vineyard were mostly high elevated and exceeded severe effect level of the Ontario guidelines (460 mg/kg for lowest effect level and 1,100 mg/kg for severe effect level) except station SM11 (this station was less affected by vine cultivation). In sediment of the vineyard, Cu exhibited low concentration compared with the Canadian sediment quality criteria (35.7 mg/kg interim fresh water sediment) and Ontario criteria (16 mg/kg for lowest effect level and 110 mg/kg for severe effect level) except in stations SM3 and SM5 which found to be the dumping site of Cu pesticide where the instrument was cleaned).

Distribution of Mn and Cu in the aquatic track was investigated at the distances of 50 m, 100 m, 500 m, and 1,000 m from the reservoir discharge points in the North and the West of the study area. The results showed that Mn and Cu concentrations in sediment slightly decreased as increasing distances correspond to the negative correlations between metal content and distance ($p<0.05$) (Figure 4.3). Also, the results showed that metal concentrations at the station SM 17 (3,052 mg Mn/kg and 21 mg Cu/kg) slightly decreased when compared with the station SM 4 (3,582 mg Mn/kg and 33 mg Cu/kg), the last station before discharged to public stream. Likewise, at the station SM 13 metal concentrations (759 mg Mn/kg and 17 mg Cu/kg) decreased when compared with the station SM 12 (1,255 mg Mn/kg and 21 mg Cu/kg). It also showed that metal containing particulate matters were settled down or precipitated in the reservoirs of the vineyard.



(a) Distribution of Mn in sediment of the North and the West direction in public stream



(b) Distribution of Cu in sediment of the North and the West direction in public stream

Figure 4.3 Mn and Cu concentration in sediment along public stream about 1 km

Sediment characteristics in the vineyard (Table 4.8) were used to explain distribution of these pollutants. The results revealed that the distribution of these pollutants was limited at the distance of 500 m from discharge point of the vineyard. Mn and Cu concentrations at this distance were equivalent to the background concentrations. The sediment characteristics of the vineyard showed high capability for retention and strong adsorption of metal in sedimentary compartment of the vineyard (with high CEC, OM and clay content) and slow water flow resulting less mobile in Mn and Cu. These metals were settled down and accumulated on the bottom of the aquatic track or reservoir before discharged to public streams. Thus the metals distributed not far away from contaminated area. Some stations at effluent discharge points found to have high Mn concentration with the level above 2,500 mg/kg (SM17, SM18 and SM19) with respect of animal excretion released into this area (Mn was found in a component of farmyard manure with high levels than Cu as shown in Table 2.5).

4.2 Potential Environmental Risk Evaluation

An ecological risk assessment is used to evaluate the potential adverse effects that human activities have on the environment (living and non-living environment) that make up ecosystems. This study, human activities were viticultural practices. The ecological risks would be evaluated by using the Hazard Quotient (HQ) equation which HQ value is based on a screening comparison of the concentrations of contaminant in environmental media with screening benchmark (a No-Adverse Effects Level). HQ is equal or greater than one for a particular contaminant that contaminant presents as a contaminant of potential ecological concern. The contaminants in this study were Mn and Cu that are the essential elements for all living organisms as well. The concentrations of both Mn and Cu which are extremely high can cause toxic effects on living organisms and ecosystem. The guidelines used for comparison with environmental concentrations in this study were shown in Table 4.10.

Table 4.10 Guidelines for ecological risks evaluation

Criteria/guidelines	Compartment	Screening level	Reference
1. Thailand Soil Quality Standard	Soil	Mn = 1,800 mg/kg dw	Pollution Control Department, Ministry of Natural Resources and Environment (2004)
2. Netherlands Quality Criteria	Soil	Cu = 36 mg/kg dw (target value)	The Netherlands Ministry of Housing, Spatial Planning and Environment
3. Ontario Sediment Quality Guideline	Sediment	Mn = 1,100 mg/kg dw (severe effect level)	Ministry of Environment and Energy of Ontario (1993)
4. Canadian Sediment Quality Guideline	Sediment	Cu = 35.7 mg/kg dw (interim freshwater sediment)	Canadian Council of Minister of Environment (CCME) (2001)

Calculations of HQ and estimations of environmental risks to potential receptors were performed in different media including soil and sediment as shown in Table 4.11 and Table 4.12 respectively;

Table 4.11 Potential environmental risk evaluation using HQ for soil in study area

Station	Description	HQ	
		Mn	Cu
R1	Background	0.33	0.44
R2	Background	0.32	0.58
S1	Contaminated	2.00	0.89
S2	Contaminated	1.89	2.31
S3	Contaminated	0.65	1.28
S4	Contaminated	1.47	0.94
S5	Contaminated	0.69	1.03
S6	Contaminated	0.83	1.25
S7	Contaminated	0.72	0.78
S8	Contaminated	0.61	1.00
S9	Contaminated	0.57	0.86
Screening Benchmark		1,800 mg/kg dw	36 mg/kg dw

Comparisons were made with literature based on soil quality standard for Mn and Cu. The results showed that the HQ values for vineyard soils in the study area were exceeded one and each of samples had Mn and Cu levels elevated above soil quality standard (Thailand permitted level of Mn in soil is not exceeded 1,800 mg/kg and the Netherlands soil quality standards for Cu is not exceeded 36 mg/kg otherwise further investigation will be required). Comparisons indicated the possibility of ecological risks at site from viticultural activities, which Mn and Cu were referred as contaminants of this site that required further investigation. However, toxicological effects on soil might be biological significance, in case Mn and Cu presented a large amount in available forms. Fortunately, with consideration of soil characteristics at site, this vineyard soil provided Mn and Cu in non-available form that can cause less toxic to living organisms.

Table 4.12 Potential environmental risk evaluation using HQ for the aquatic track

Station	Description	HQ	
		Mn	**Cu**
SM1	Upstream	2.88	0.69
SM2	Upstream	2.94	0.64
SM3	Upstream	1.53	4.33
SM4	Upstream	3.26	0.92
SM5	Upstream	1.52	3.67
SM6	Upstream	4.08	1.00
SM7	Upstream	1.51	0.64
SM8	Upstream	1.66	0.75
SM9	Upstream	1.13	0.69
SM10	Upstream	1.40	0.58
SM11	Upstream	0.48	0.64
SM12	Upstream	1.14	0.58
SM13	Downstream (50 m)	0.69	0.47
SM14	Downstream (100 m)	0.81	0.67
SM15	Downstream (500 m)	0.49	0.58
SM16	Downstream (1,000 m)	0.48	0.33
SM17	Downstream (50 m)	2.77	0.58
SM18	Downstream (100 m)	2.78	0.64
SM19	Downstream (500 m)	2.37	0.58
SM20	Downstream (1,000 m)	0.69	0.50
Screening Benchmark (for protection of aquatic life)		1,100 mg/kg dw (severe effect level)	35.7 mg/kg dw

HQs for sediment samples were calculated based on Sediment Quality Guidelines for the protection of aquatic life using severe effect level of Ontario standard for Mn evaluation and interim freshwater sediment of Canadian standard for Cu. The purposed values obtained from the background concentration of the study area and the limit of the standard, were also used for HQ determination (Table 4.12). The results showed that Mn concentrations of sediments at upstream stations were high elevated above severe effect level (except SM11 where this site associated very small with vine cultivation). At downstream stations, the HQ of three of them found to be greater than 1 (SM17, SM18 and SM19) with respect of the evidence of cattle living nearby. For Cu, three of upstream stations (station located in the vineyard) had HQ values greater than 1.

The HQ values of vineyard sediment that were exceeded one showed elevated concentration above screening benchmark in both Mn and Cu. There was a potential for adverse ecological effect associated with viticultural practices. The environmental investigation and risk elimination are required. This investigation was also found that Mn and Cu were less distribution as exhibition of sediment characteristics with high capability for metal complexation. The ecological risks were presented only in sediment of vineyard. However, Mn and Cu are essential elements for normal growth and its toxicity presented low. Such concentrations can affect aquatic organisms or microorganisms when the environmental compartments are exposed in large amount of available metal and with longer period of time.

4.3 Linkages of Mn and Cu in Environmental Compartments of Study Area

Viticultural practices can encourage Mn and Cu contributions to ecological systems. The initial distribution can occur through both fertilizing and spraying some of agrochemicals. The pollutants can be transported and mobilized between environmental compartments as shown in Figure 4.4.

From the study, Mn and Cu entered to environmental systems through both direct and indirect processes. Spraying some of pesticides and fertilizers caused atmospheric contamination directly, in term of fine suspended particulate matters. The suspended particulate matters can be transported in air and deposit as atmospheric fall out to the other media by wet and dry deposition. The study presented that soil environment received Mn and Cu from direct addition (fertilizing) and deposition process. Consequently, the soil concentrations exhibited high levels above background levels in some stations. The contamination in soil is then potential in associated with vine (dominate species in study area) and crop products in term of plant adsorption and transportation to be accumulated in edible parts of plant (grape fruit) connected to food chain by human ingestion.

Mn and Cu concentrations in vineyard sediments indicated that the distribution of pollutants to sedimentary compartment via runoff can be leached from soil into particulate matters by rain. The particulate matters then runoff to deposit onto a bottom of the aquatic track. At the end they were accumulated in this compartment which connected to aquatic organisms by direct contact and ingestion of sediment.

Other exposure pathways derived from this study included inhalation of metal adsorbed fine particles and vapor in the air during and after fertilizer spraying and tilling. Direct exposure was ingestion of agrochemical during application.

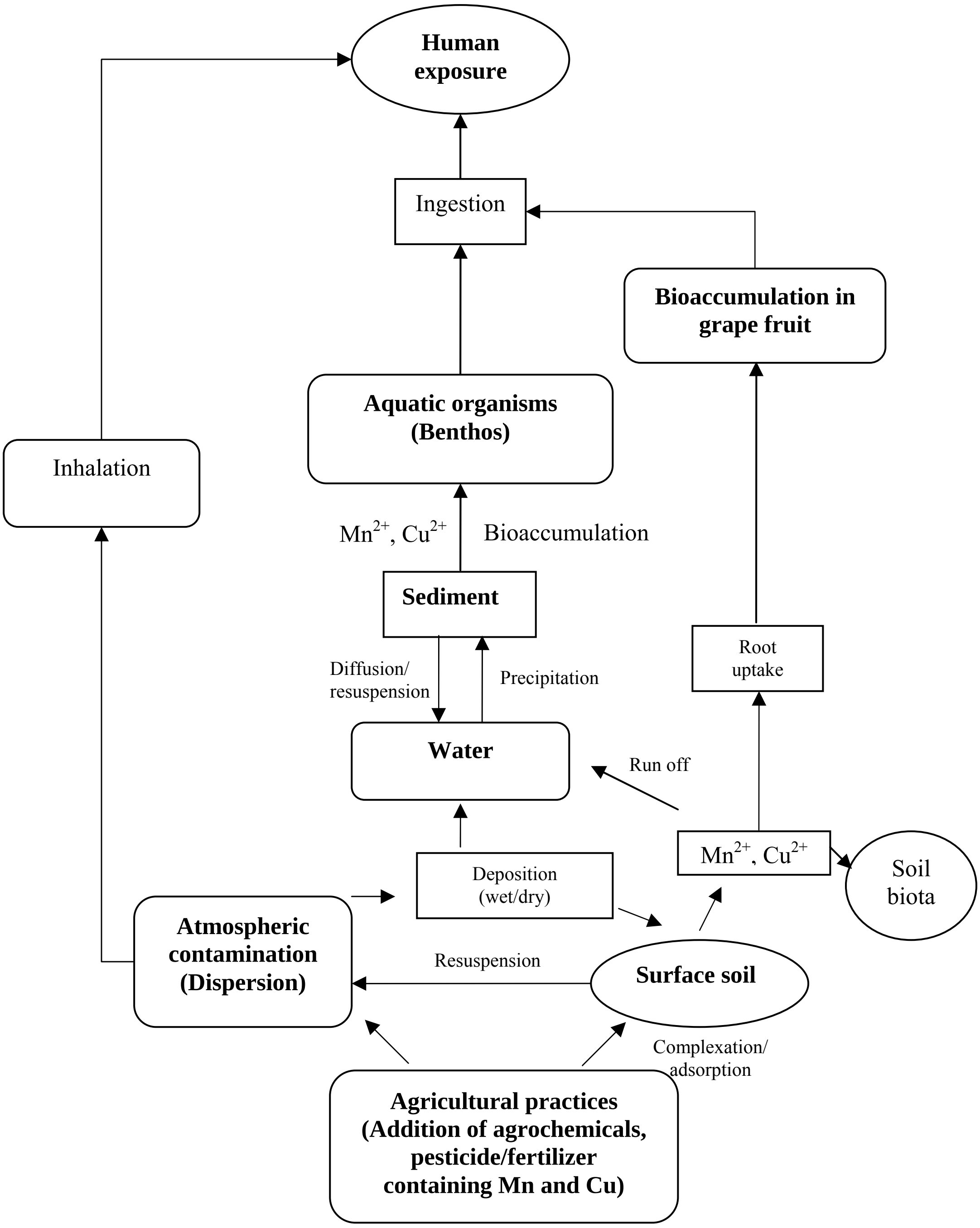


Figure 4.4 Linkages among contaminated environmental compartments and their exposure pathways in vineyard of the study area

CHAPTER V
CONCLUSIONS AND RECCOMMENDATIONS

5.1 Conclusions

Distribution of Mn and Cu contaminated from viticultural practices in a GAPs vineyard were investigated. The study was focused on surface soil, sediment and crop products (grape fruit) grown in the study area.

5.1.1 The level of Mn and Cu in the vineyard soil resulting from the contamination of agrochemical applications were elevated above background concentrations and standards. The Mn and Cu contents varied from 1,029 to 3,578 mg/kg dw and 28 to 83 mg/kg dw, respectively. The correlation between metal concentrations in soil and period of cultivation were found in most of sampling stations.

5.1.2 From the results, there is no correlation between heavy metal concentration in vineyard soil and type of grape and rate of application. The metal concentration in vineyard soil was affected by period and rate of chemical application, metal component and quantity in chemicals and historical practices of the study site.

5.1.3 There was no correlation between metals in soil and bioaccumulation in crop product (grape fruit). The bioaccumulation was very low and associated with physical and chemical characteristics of vineyard soil which provide metal in non bioavailable form or less soluble for root adsorption (neutral pH, high CEC and OM and moderate clay content). Furthermore, Mn and Cu concentration in grape fruit might be affected by species or varieties of plant and metabolic process in bioaccumulation.

Mn and Cu concentrations in grape fruit were ranged from 0.79 to 3.82 mg/kg ww and from 0.39 to 1.82 mg/kg ww, respectively, such concentrations were less than the maximum permitted concentration for Cu (20 mg/kg ww). There was no limited or standard for Mn in food but concentration exhibited low level.

5.1.4 In the sediment, Mn and Cu concentration ranged from 526 to 4,490 mg/kg dw and 21 to 36 mg/kg dw, respectively. These contents were elevated above background levels. This indicated the contamination of Mn and Cu in this compartment. The Cu concentration was found below the standard for protection of aquatic life because Cu is more strongly bound to organic and clay content than Mn.

5.1.5 Distribution of Mn and Cu in aquatic environment was also assessed at a distance of 50 m, 100 m, 500 m and 1,000 m from the effluent discharge point of vineyard. It showed that their transportations do not exceed the distance of 500 m due to high capability for the retention of metal in sediment. It was also found that Mn and Cu slightly decreased as increasing distances.

5.1.6 Potential environmental risks for soil and sediment were evaluated. It was found that both soil and sediment were at risk (HQ > 1). This indicated a possibility of ecological risks of the site from viticultural activities. However, Mn and Cu mainly present in a form of non bioavailability, which had less toxic effects to living organisms.

5.2 Recommendations

5.2.1 The data provided only the information of Mn and Cu in term of total concentrations and can not be evaluated the potential phytotoxicity directly in a form of bioavailability. Identification of bioavailability should be performed through exchangeable form of metals that presented to be available for plant uptake which can be then explained potential toxic as well.

5.2.2 Bioaccumulation of Mn and Cu in aquatic organisms (i.e. bivalve, aquatic plants, fish) and soil biota should be investigated because of the presence of their high concentrations in sediment and soil.

5.2.3 Other agricultural areas should be investigated in order to provide based line data of metal contamination from agricultural practice of Thailand. The study should include other metals such as As, Cd, Cr, Pb and Zn.

5.2.4 Besides ecological risk evaluation, health risk assessment of workers and residents in the vicinity of the vineyard who may expose these contaminants should be investigated as well.

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APPENDIX

APPENDIX A
DATA FROM THE EXPERIMENT

Table A-1 The instrument blank of FAAS and method blank of Nitric/Hydrochloric acid digestion

Instrument blank

Blank	**Metal (ppm)**	
	Mn	**Cu**
Deionized water	N.D	N.D
Deionized water	N.D	N.D
Deionized water	N.D	N.D
N.D	<0.001	

Method blank (Nitric/Hydrochloric acid digestion)

Blank	**Metal (ppm)**	
	Mn	**Cu**
Blank1	N.D	N.D
Blank2	0.003	N.D
Blank3	0.007	N.D
Blank4	0.013	N.D
Blank5	N.D	N.D
Blank6	N.D	N.D
Blank7	N.D	N.D
Blank8	N.D	N.D

Table A-2 Mn and Cu concentration in surface soils at various stations (December, 2006)

Stations	Replicates	Total concentrations (mg/kg dw)	
		Mn	Cu
R1	1	600	16.7
	2	574	15.6
	3	627	16.8
	Mean	600	16.4
R2	1	562	21.1
	2	572	21.1
	3	579	21.1
	Mean	571	21.1
S1	1	3656	31.6
	2	3494	31.6
	3	3586	32.0
	Mean	3579	31.7
S2	1	3246	83.3
	2	3447	81.7
	3	3531	85.0
	Mean	3408	83.3
S3	1	1194	46.5
	2	1131	45.8
	3	1165	46.8
	Mean	1163	46.4
S4	1	2618	33.7
	2	2637	34.0
	3	2665	35.3
	Mean	2640	34.3
S5	1	1192	36.2
	2	1260	36.3
	3	1278	37.2
	Mean	1243	36.6
S6	1	1498	44.0
	2	1558	44.1
	3	1435	45.9
	Mean	1497	44.7

Table A-2 Mn and Cu concentration in surface soils at various stations (December, 2006) (Continued)

Stations	Replicates	Total concentrations (mg/kg dw)	
		Mn	Cu
S7	1	1305	28.2
	2	1297	27.4
	3	1281	27.7
	Mean	1294	27.8
S8	1	1146	35.0
	2	1055	35.5
	3	1076	36.2
	Mean	1092	35.6
S9	1	1112	33.4
	2	975	30.8
	3	999	28.9
	Mean	1029	31.0

Table A-3 Mn and Cu concentration in sediment at various stations (December, 2006)

Station	Total concentration (mg/kg)			
	Mn	Average	Cu	Average
SM1	3201 3138	3170	26.8 23.3	25
SM2	3378 3096	3237	22.6 22.5	23
SM3	1684 1690	1687	158 154	156
SM4	3618 3546	3582	32.6 32.6	33
SM5	1685 1652	1669	131 133	132
SM6	4574 4405	4490	35.8 35.7	36
SM7	1667 1660	1664	22.9 22.6	23
SM8	1823 1827	1825	28.2 25.6	27
SM9	1266 1227	1247	24.1 25.1	25
SM10	1563 1509	1536	22.1 19.5	21
SM11	525 527	526	23.5 22.7	23
SM12	1251 1260	1256	20.8 20.8	21
SM13	775 744	760	17.5 16.7	17
SM14	918 871	895	23.9 23.8	24
SM15	537 532	535	21.2 21.2	21
SM16	538 519	529	12.5 12.1	12

Table A-3 Mn and Cu concentration in sediment at various stations (December, 2006) (Continued)

Station	Total concentration (mg/kg)			
	Mn	Average	Cu	Average
SM17	3034 3069	3052	20.5 21.6	21
SM18	3084 3029	3057	22.9 23.5	23
SM19	2550 2657	2604	20.5 20.5	21
SM20	764 758	761	17.5 17.8	18

Table A-4 Mn and Cu concentration in grape fruit at the same site as was done for soil (March, 2007)

Stations	Concentration (mg/kg ww)			
	Mn	Mean	Cu	Mean
S1	2.685 1.795 2.452	2.311	0.64 0.776 0.533	0.650
S2	1.621 1.558 1.647	1.609	1.174 1.094 1.133	1.134
S3	0.824 0.869 0.927	0.873	1.786 1.806 1.864	1.819
S4	1.094 1.076 1.106	1.092	0.466 0.423 0.431	0.440
S5	1.404 1.311 1.28	1.332	0.402 0.384 0.376	0.387
S6	2.067 2.067 2.324	2.153	0.807 0.884 0.706	0.799
S7	0.807 0.798 0.798	0.801	0.497 0.485 0.461	0.481
S8	3.527 3.914 4.021	3.821	0.494 0.513 0.476	0.494
S9	0.816 0.782 0.782	0.793	0.405 0.396 0.396	0.399

APPENDIX B
STATISTICAL ANALYSIS

(1) Soil

Table B-1 Statistic analysis of metal concentrations in surface soil of vineyard at various stations

Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Mn	.266	33	.000	.807	33	.000
Cu	.226	33	.000	.803	33	.000

NPar Test

Mann-Whitney Test

Mn

Descriptive Statistics

	Group	N	Mean Rank
Mn	Contaminated soil	27	20.00
	Background soil	6	3.50
	Total	33	

Test Statistics

	Mn
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-3.781
Asymp. Sig.	.000
Exact Sig.	.000

Grouping Variable: background site

Cu

Descriptive Statistics

	groups	N	Mean Rank
Cu	Contaminated soil	27	20.00
	Background soil	6	3.50
	Total	33	

Test Statistics

	Cu
Mann-Whitney U	.000
Wilcoxon W	21.000
Z	-3.783
Asymp. Sig.	.000
Exact Sig.	.000

Grouping Variable: contaminated site

Table B-2 Correlation between soil metal concentrations and vine age

Correlation

Mn

Descriptive Statistics

	Mean	Std. Deviation	N
Mn	1882.851	995.0121	27
Vine age	8.67	4.132	27

Nonparametric Correlations

			Mn	vineage
Kendall's tau_b	Mn	Correlation Coefficient	1.000	.746
		Sig.	.	.000
	Vine age	Correlation Coefficient	.746	1.000
		Sig.	.000	.
Spearman's rho	Mn	Correlation Coefficient	1.000	.893
		Sig. (2-tailed)	.	.000
	Vine age	Correlation Coefficient	.893	1.000
		Sig.	.000	.

Correlation is significant at the 0.01 level (2-tailed).

N = 27

Cu

Descriptive Statistics

	Mean	Std. Deviation	N
Cu	41.2630	16.27259	27
Vine age	8.67	4.132	27

Nonparametric Correlations

			Vine age	Cu
Kendall's tau_b	Vine age	Correlation Coefficient	1.000	.298
		Sig.	.	.041
	Cu	Correlation Coefficient	.298	1.000
		Sig.	.041	.
Spearman's rho	Vine age	Correlation Coefficient	1.000	.390
		Sig.	.	.044
	Cu	Correlation Coefficient	.390	1.000
		Sig.	.044	.

Correlation is significant at the 0.05 level (2-tailed).

N = 27

(2) Grape

Table B-3 Statistic analysis of metal concentrations in grape of vineyard at various stations

Tests of Normality

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Mn grape	.186	27	.017	.826	27	.000
Cu grape	.262	27	.000	.742	27	.000

Npar

Mann-Whitney Test

Test difference between Mn and Cu concentration in grape

Test Statistics

Mann-Whitney U	103.000
Wilcoxon W	481.000
Z	-4.524
Asymp. Sig.	.000
Exact Sig.	.000

Grouping Variable: metal

Table B-4 Correlations between metal concentrations in soil and grape

Correlation

Mn

Descriptive Statistics

	Mean	Std. Deviation	N
Mn grape	1.6427	.96559	27
Mn soil	1882.8519	995.01213	27

Nonparametric Correlations

			Mn grape	Mn soil
Kendall's tau_b	Mn grape	Correlation Coefficient	1.000	.263
		Sig.	.	.055
	Mn soil	Correlation Coefficient	.263	1.000
		Sig.	.055	.
Spearman's rho	Mn grape	Correlation Coefficient	1.000	.325
		Sig.	.	.098
	Mn soil	Correlation Coefficient	.325	1.000
		Sig.	.098	.

N = 27

Cu

Descriptive Statistics

	Mean	Std. Deviation	N
Cu grape	.7336	.45651	27
Cu soil	41.2630	16.27259	27

Nonparametric Correlations (a)

			Cu grape	Cu soil
Kendall's tau_b	Cu grape	Correlation Coefficient	1.000	.372
		Sig.	.	.007
	Cu soil	Correlation Coefficient	.372	1.000
		Sig.	.007	.
Spearman's rho	Cu grape	Correlation Coefficient	1.000	.571
		Sig.	.	.002
	Cu soil	Correlation Coefficient	.571	1.000
		Sig.	.002	.

Correlation is significant at the 0.01 level (2-tailed).

N = 27

Table B-5 Correlations between grape metal concentrations and soil pH

Correlation

Mn

Nonparametric Correlations

			Mn grape	pH
Kendall's tau_b	Mn grape	Correlation Coefficient	1.000	-.568
		Sig.	.	.000
	pH	Correlation Coefficient	-.568	1.000
		Sig.)	.000	.
Spearman's rho	Mn grape	Correlation Coefficient	1.000	-.720
		Sig.	.	.000
	pH	Correlation Coefficient	-.720	1.000
		Sig.)	.000	.

Correlation is significant at the 0.01 level (2-tailed).

Listwise N = 27

Cu

Nonparametric Correlations

			pH	Cu grape
Kendall's tau_b	pH	Correlation Coefficient	1.000	-.483
		Sig.	.	.001
	Cu grape	Correlation Coefficient	-.483	1.000
		Sig.	.001	.
Spearman's rho	pH	Correlation Coefficient	1.000	-.653
		Sig.	.	.000
	Cu grape	Correlation Coefficient	-.653	1.000
		Sig.	.000	.

Correlation is significant at the 0.01 level (2-tailed).

N = 27

(3) Sediment

Table B-6 Statistic analysis of metal concentrations in sediment inside vineyard

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Mninsides	24	525.26	4574.05	2157.1226	234.63752	1149.48440
Cuinsides	24	19.50	157.70	45.2208	9.30026	45.56180

Table B-7 Statistic analysis of metal concentrations in sediments outside vineyard at public streams about 1 km

Mn

Tests of Normality

	Kolmogorov-Smirnov (a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
North	.301	8	.031	.830	8	.059
West	.321	8	.015	.701	8	.002

a Lilliefors Significance Correction

North Direction

Correlation

		North	Distance
North	Pearson Correlation	1	-.830
	Sig.		.011
	N	8	8
Distance1	Pearson Correlation	-.830	1
	Sig.	.011	
	N	8	8

Correlation is significant at the 0.05 level (2-tailed).

West Direction

Nonparametric correlation

			Distance	West
Kendall's tau_b	Distance1	Correlation Coefficient	1.000	-.772
		Sig.	.	.011
		N	8	8
	West	Correlation Coefficient	-.772	1.000
		Sig.	.011	.
		N	8	8
Spearman's rho	Distance1	Correlation Coefficient	1.000	-.878
		Sig. (2-tailed)	.	.004
		N	8	8
	West	Correlation Coefficient	-.878	1.000
		Sig.	.004	.
		N	8	8

* Correlation is significant at the 0.05 level (2-tailed).

Cu

Tests of Normality

	Kolmogorov-Smirnov (a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
North	.210	8	.200(*)	.897	8	.270
West	.231	8	.200(*)	.918	8	.416

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

North Direction

Correlations

		North	Distance1
North	Pearson Correlation	1	-.685
	Sig.		.061
	N	8	8
Distance1	Pearson Correlation	-.685	1
	Sig.	.061	
	N	8	8

West Direction

Correlations

		Distance1	West
Distance1	Pearson Correlation	1	-.888
	Sig.		.003
	N	8	8
West	Pearson Correlation	-.888	1
	Sig.	.003	
	N	8	8

Correlation is significant at the 0.01 level (2-tailed).

APPENDIX C

PHYSICAL AND CHEMICAL ANALYSIS OF SOIL

Soil pH

(pH H_2O)

Equipments

1. pH meter
2. 50 mL Beaker
3. Stirring rod
4. 10 mL Measuring cylinder

Reagents

1. Standard buffer solution pH 4 and 7
2. Deionized water

Procedures

1. Weight 10 g of soil into a 50 mL beaker.
2. Add 10 mL of deionized water. Thoroughly mix the solution with a stirring rod and allow it to sit for a minimum of 30 minutes and a maximum of 2 hours.
3. The pH meter should be adjusted pH reading with a pH 7 and 4 of standard buffer solutions before being used to measure pH of soil solution.

Organic Matter
(Walkley and Black Method)

Equipments

1. 250 mL Erlenmeyer flasks
2. 10 mL pipette
3. 25 and 50 mL Measuring cylinder
4. 50 mL burette
5. Analytical balance

Reagents

1. Concentrated of sulfuric acid (H_2SO_4)
2. Potassium dichromate ($K_2Cr_2O_7$) 1 N
3. Ferrous Ammonium Sulphate [$Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$] 0.5 N
4. O-phenanthroline indicator 0.025 M

Procedures

1. Weigh 1 g of dried soil and transfer to a 250 mL Erlenmeyer flask.
2. Add 10 mL of 1 N $K_2Cr_2O_7$ by means of a pipette.
3. Add 15 mL of concentrated H_2SO_4 and swirl gently to mix. Avoid excessive swirling that would result in organic particles adhering to the sides of the flask out of the solution.
4. Place the flasks on an insulation pad and let stand 30 minutes.
5. Dilute the suspension with about 50 mL of deionized water and allow to cool.
6. Add 5 drops of O-phenanthroline indicator. The indicator should be added just prior to titration to avoid deactivation by adsorption onto clay surfaces.
7. Titrate with 0.5 N Ferrous Ammonium Sulphate to a burgundy endpoint. The color of the solution at the beginning is yellow-orange to dark green, depending on the amount of unreacted $Cr_2O_7^{2-}$ remaining, which shifts to a turbid gray before the endpoint and then changes sharply to a wine red at the endpoint. This will eliminate uncertainty in determining the endpoint by color change.

8. Run a reagent blank following the above procedure without soil. The reagent blank is used to standardize the Ferrous Ammonium Sulphate solution daily.

9. Calculate organic carbon and organic matter percentages:

a. Percentage easily oxidizable organic carbon:

$$\% \text{ Organic carbon} = \frac{(B-T)N}{B} \times \frac{100}{77} \times 3 \times \frac{100}{10^3} \times \frac{10}{W}$$

B = mL of Ferrous Ammonium Sulphate solution used to titrate blank

T = mL of Ferrous Ammonium Sulphate solution used to titrate sample

N = Concentration of Potassium dichromate (N)

W = Weight of sample (g)

b. Percentage of organic matter (OM):

$$\% \text{ Organic matter} = \frac{\% \text{ Organic carbon}}{0.58} = \% \text{ Organic carbon} \times 1.724$$

Cation Exchange Capacity (CEC)
(Ammonium Replacement Method)

Equipments

1. 125 mL Erlenmeyer flask
2. Buchner funnels vacuum flasks and source of vacuum
3. 500 mL Filtering flask
4. Whatman No. 42
5. 100 mL Volumetric flask
6. 800 mL Kjeldahl flask
7. 50 mL Burette
8. pH meter
9. Distillation equipment
10. Analytical balance

Reagents

1. Deionized water
2. Ammonium acetate (NH_4OAc)1 N pH 7
3. Ethanol, 95%
4. Hydrochloric acid, 0.1 N
5. Ammonium oxalate [$(NH_4)_2C_2O_4 \cdot H_2O$], 10%
6. Ammonium hydroxide (NH_4OH), 50%
7. Ammonium chloride (NH_4Cl_2), 1 N
8. Ammonium chloride (NH_4Cl_2), 0.25 N
9. Silver nitrate ($AgNO_3$), 0.01 N
10. Soduim hydroxide (NaOH), 40%
11. Boric acid (H_3BO_3), 3%
12. Mixed indicator (Bromo glycerol and Methyl red)
13. Sodium Chloride (NaCl), 10%

Procedures

1. Weigh 5-10 g of dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) soil into a 125 mL Erlenmeyer flask. Add 60 mL of ammonium acetate solution and close the flask, and then swirl gently to mix and let stand overnight.

2. Connect a 1 L vacuum extraction flask to a Buchner funnel fitted with a Whatman No. 42. Moisten the filter paper with 2 mL deionized water

3. Transfer the soil suspension into the Buchner funnel and leach the sample with 175 mL of 1 N ammonium acetate. The soil extract may be analyzed for extractable K, Ca, Mg, and Na.

4. Leach the soil sample in the Buchner funnel by leaching with 1 N of Ammonium chloride 4 times and 0.25 N of Ammonium chloride 1 time after that rinse with 95 % of ethanol to remove chloride and discard the leachate.

5. Rinse the soil sample with 10% of NaCl , Na^+ to replace the exchangeable ammonium. Bring leachate to a final volume of 225 mL volumetric flask using deionized water.

6. Pour the soil solution in step 5 into Kjeldahl flask and add a little amount of Pumice stone.

7. This determination can also be made using the Kjeldahl distillation method. Add 25 mL of 40% NaOH into soil solution in Kjeldahl flask to increase pH of soil solution to remove NH_4^+.The vapor of NH_3 from distillation is trap in 50 mL of 3% boric acid.

8. Add 5 drops of mixed indicator into solution from step 7 until solution change color from violet to green. Titrate with 0.1 N Hydrochloric aid to a burgundy endpoint. The color of the solution at the beginning is green to violet at the endpoint.

9. Run a reagent blank following the above procedure without soil.

10. Calculate CEC

$$\text{CEC (cmol/kg)} = \frac{(A-B)N \times 100}{W}$$

A = HCl solution used to titrate sample (mL)

B = HCl solution used to titrate blank (mL)

N = Concentration of HCl solution (N)

W = Weight of sample (g)

Soil Texture
(Pipette method)

Equipments

1. Beaker (glass and plastic)
2. Cover glass
3. Measuring cylinder
4. Centrifuge
5. Centrifuge tube
6. Stirring rod
7. Policeman
8. Plunger
9. Aluminum can
10. Pipette apparatus
11. Hot plate.
12. Hood
13. Hot air oven
14. Mechanical stirrer
15. Time piece
16. Thermometer
17. Sieve shaker
18. Water bath
19. Desiccator

Chemicals and preparation

1.Preparation of calgon solution

Mix 35.7 g of $(NaPO_3)_6$ and 7.94 g of (Na_2CO_3) with 1 liter of distilled water to make a solution

2. H_2O_2 (50%)

3. 1 N NaOAc pH 5

Add 82 g of NaOAc to 800 mL of deionized water and add 27 mL of glacial acetic acid adjust volume to 1,000 mL with deionized water and adjust solution pH to 5 by adding acetic acid

4. Sodium citrate solution 0.3 M

Add 88 g of sodium citrate and adjust volume to 1 L by adding deionized water and thoroughly mix a solution

5. Sodium bicarbonate solution 1 M

Add 84 g of sodium bicarbonate and adjust volume to 1 L by adding deionized water and thoroughly mix a solution

6. Pure sodium dithiosulphate ($Na_2S_2O_4$)

Procedures

1. Weigh 10 g of soil sample with through the sieve nest with a 2 mm sieve and place the weighted samples into 600 mL beaker and add 5-10 mL of hydrogen peroxide to each beaker. The samples were analyzed in replicates of two of each sample. Add more H_2O_2 when the reaction subsides, rinsing the sides if necessary. After that the samples were refluxed on hot plate at 50-60 °C for 1-2 hour with out boiling. If the samples have high organic contents should be add hydrogen peroxide again. Repeat steps until most organic matter has been destroyed, as determined by color of sample and rate of reaction.

2. The replicates of two soil sample from 1, bring one beaker for dry at 105°C for 24 hour until weight were constant and use for calculation **(a)**.

3. Add about 10 mL of Calgon solution in another one beaker. Stir for about 15 minutes this will disperse the aggregates in the soil.

4. Separation of the sand fractions

4.1 Wet the screen of a 53 µm sieve with water.

4.2 Slowly pour the solution in step 3 through the sieve. Collect the solution passing through the sieve in a 1,000 L beaker.

4.3 Collect the sand particles remaining on the 53 µm sieve into a dish. Using a distilled water rinse stream, pass this fraction through a stack of sieves with sizes between 53 µm and 2 mm (arranged coarsest on top, finest on the bottom). Shake the sieves for approximately 5 minutes. Add any solution passing through the 53 μm sieve to the 1,000 mL beaker containing the rinsing from step 2.

4.4 Label, weight, and record the mass of a aluminum drying dish for each sieve used. Transfer the soil particles retained on each sieve to the appropriate weighting dish. Rinse sieve to ensure allow particles are transferred.

4.5 Oven dry the sand fraction captured on each sieve.

4.6 Weigh and record the mass of the oven-dried sand fraction and dishes.

4.7 Calculate the mass of soil collected on each sieve **(b)**.

5. Analysis of the Clay and Silt Fraction

5.1 Label weight and record the weight of three aluminum drying cans.

5.2 Place the dispersed mixture which has been allowed to soak into a 1,000 mL graduated cylinder. Rinse beaker thoroughly with distilled water to ensure that all soil particles are in the cylinder.

5.3 Add 10 mL of dispersing agent to the cylinder.

5.4 Add sufficient distilled water to bring the volume in the solution to 1,000 mL.

5.5 Cover the cylinder with lab film and briefly mix solution by shaking the cylinder.

5.6 Allow the solution to equilibrate overnight.

5.7 Mark the point which is 5 cm below the top of the liquid surface.

5.8 Shake the cylinder for at least two minutes to ensure that the soil is dispersed throughout the solution. Record the time that shaking is stopped.

5.9 Gently insert the 20 mL pipette to the marked depth of 5 cm in the cylinder's liquid.

5.10 Using a controlled vacuum, withdraw 25 mL of solution in approximately 12 seconds.

5.11 Gradually remove the full pipette from the solution.

5.12 Discharge the liquid from the pipette into a previously weighted and numbered aluminum drying dish.

5.13 Using a small amount of distilled water, rinse the pipette into the drying dish.

5.14 Oven-dry the samples at 105±5°C until the water evaporate.

5.15 Cool the drying dishes in a desiccator and then weigh and record weight **(c)**.

6. Calculate % sand, % silt, % clay

Weight of dry soil = a g

Weight of sand = b g

% sand = $\frac{100 \times b}{a}$ %

Pipette soil solution = 20 mL

Weight of clay = c g

% clay = $\frac{1{,}000 \times c}{20a} \times 100$ %

= $\frac{5{,}000 \times c}{a}$ %

Amount of calgon must be removed from weight of clay before calculated

% silt = 100-(% sand + % clay)

BIOGRAPHY

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