HEAVY METALS SPECIATION IN AGRICULTURAL SOILS

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This project is submitted in partial fulfillment of the requirements for the degree of Bachelor of Science with Honours.

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DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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ABSTRACT

Heavy metals in soils has been occurring naturally for million of years due to the natural weathering of the parent rocks and has increased due to other factors including application of agricultural materials such as fertilizer. Soil samples were collected from the pineapple farm in Kampung Meranek, Kota Samarahan. Soil characteristics namely pH, total organic matter and soil textural classes were determined. Total metals concentration of cadmium, chromium and mercury was determined. The extractability of the heavy metals with single extraction and sequential extraction between soils with fertilizer application and without fertilizer application was compared. The results showed that the highest total heavy metals concentration was chromium, followed by cadmium and mercury. The order of extractability of heavy metals in sampling sites was: Cd > Cr > Hg. The soils with fertilizer application showed higher heavy metals concentration compared to soil without fertilizer application. Statistical analysis showed no significant difference (P>0.05) for cadmium, chromium, and mercury concentrations in soils with fertilizer application and soils without fertilizer application. However, statistical analysis showed significant difference (P<0.05) for cadmium concentrations in different fractions in soils with fertilizer application. Chromium and mercury concentrations in different fractions showed significant difference in soils without fertilizer application but no significant difference in soils with fertilizer application. The sequential extraction procedure showed that all the heavy metals in both soils with fertilizer and without fertilizer application were dominant in the oxidizable fraction.

Key word: heavy metals, speciation, extractability, single extraction, sequential extraction, soil.

ABSTRAK


Kata kunci: Logam-logam berat, spesiasi, kebolehekstrakan, pengekstrakan berasingan, pengekstrakan berurutan, tanah.
CHAPTER ONE
INTRODUCTION

1.0 Introduction

Contamination of the terrestrial environment by heavy metals has been occurring for million of years due to the natural weathering of the parent rocks which precipitate metals into the terrestrial system (Ward, 2007). This contamination has vastly increased through various factors including metalliferous mining and smelting, biosolid disposal, fossil fuel combustion, traffic-related emissions, waste disposal, atmospheric deposition, discharge of wastewater on land (Verma et al., 2006), and agricultural and horticultural materials (Ward, 2007) such as fertilizers application. Many potentially adverse contaminants are necessary for agricultural production at first but become hazardous when they occur in excess.

In agricultural activities, fertilizers are required to supply nutrients for maintaining the level of soil fertility and improving both the quality and quantity of crops produced (Ramachandra, 2006). In addition, applying fertilizers to the soils may also change the speciation and bioavailability of heavy metals in soils and also alter soil properties such as pH and surface charge (Chen, 2000).

Soil plays an important role for the adverse heavy metals transport and storage. On the other hand, the interactions of soil-plant root-microbes also plays major role in regulating heavy metal movement from soil to the other parts of crops (Islam et al., 2007). According to Chen (2000), the major trace elements usually found in contaminated soils were cadmium, copper, chromium, nickel, lead and zinc.
The total metal concentration of an element usually used to determine the maximum levels of a polluting element in an environment sample in order to evaluate the element toxicity. However, total metal concentration does not provide information on the fate of the elements in terms of their interaction with soils, their bioavailability or its resultant toxicity. On the other hand, changes in speciation of an element can give effect to the toxicity of a metal. Thus, heavy metals speciation in agricultural soils is important to give information concerning their chemical form in order to assess the environmental impact of the heavy metals (Christie, 1995).

1.1 Statement of Problems

Soil pollution due to toxic heavy metal accumulation is a very serious and widespread environmental problem due to their persistent and non-biodegradable properties (Cuong and Obbard, 2006). The increasing accumulation of heavy metals in the environment mainly agricultural soils may lead to harmful human health effects (Melaku et al., 2005).

When fertilizers are applied to crops, besides nutrients, some trace metals in the fertilizers are also taken up by the plants (Chen, 2000). Consequently, those excess trace metals become toxic to human who consumes large quantities of vegetables and fruits. Agricultural land usually applied both types of organic and chemical fertilizer to prepare good condition of soil for the plant growth and development.

The agricultural land in Kota Samarahan specifically in the area of pineapple development project in Kampung Meranek in the division of Kota Samarahan applied NPK fertilizer with N: P\textsubscript{2}O\textsubscript{5}: K\textsubscript{2}O ratio of 15: 15: 15 besides Behn Meyer compound fertilizer,
Supreme Green and ABF Granule Urea (12-12-17-2+ Trace Elements). These fertilizers were added to the soil for the better uptake by the plants.

However, impurities in the fertilizers such as Cd, Cu, Cr, Pb, Zn and Ni can cause harmful effects when they accumulate in soils (Curtis and Smith, 2002). Since use of fertilizers is a repetitive practice, it is necessary to consider cumulative changes over decades of applications. Hence, it is crucial to study the soil used for agricultural purpose in order to assess the safety of the soil. Therefore, the physico-chemical properties of the metal concerned in the soil and the media it is entering must be understood first in order to fully understand the fate of heavy metal contamination in the soil (Ward, 2007).

1.2 Objectives

The objectives of this study were to:

a) characterize the soil samples with fertilizer and without fertilizer application for pH, organic matter contents, textural classes, and total metal concentrations i.e. Cadmium (Cd), Chromium (Cr), and Mercury (Hg).

b) evaluate one sequential extraction procedure and single extraction method in order to compare the extractability of metals (Cd, Cr, and Hg) in soils applied with fertilizer and soils without fertilizer application.

c) compare heavy metals concentrations in soil applied with fertilizer and soils without fertilizer application using statistical analysis.

d) compare heavy metals concentration in different fractions produced from sequential extraction using statistical analysis (one-way ANOVA).
CHAPTER TWO
LITERATURE REVIEW

2.1 Heavy Metals

A large number of elements are often called as heavy metals when present in excessive amounts or when known to be basically hostile to living organisms at any level while trace elements are when the elements viewed as beneficials (Csuros and Csuros (2002). According to Csuros and Csuros (2002), heavy metals is a metal with an atomic density larger than 5 g/cm³, i.e., specific gravity >5.

Daintith (2004) defined heavy metal as a metal with a high relative atomic mass, such as cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), zinc (Zn), arsenic (As), chromium (Cr), and others. Heavy metals can be toxic when they are excessively present in soils. They are also often associated with soil pollution toxicity activities. Certain heavy metals such as zinc, cadmium, and lead are naturally occurring in the soils but their concentrations in agricultural soils may increase due to fertilizer use over long periods of time (Rogowski et al., 1999). Curtis and Smith (2002) stated that physical process and chemical process are the determinants of transport and fate of heavy metals in fertilizer applied to soil.

Heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants, particularly in areas with high anthropogenic pressure. Their presence in the atmosphere, soil and water, even in traces can cause serious problems to all organisms, and heavy metal bioaccumulation in the food chain especially can be highly dangerous to human
health. Heavy metals enter the human body mainly through two routes namely: inhalation and ingestion (Alloway, 1990).

Some of the worst contaminants making their way into the country's food come from the soil in which it is grown. In a study published earlier this year, researchers at the Guangdong Institute of Ecology found excessive levels of cadmium and mercury in Chinese cabbage grown in Foshan, a major manufacturing center in southern China. A study of crops grown in the central city of Chongqing found excessive lead and cadmium levels in vegetables at 20 sites (Zamiska and Spencer, 2007).

2.1.1 Cadmium (Cd)

Cadmium is a rare metal and is the 67th abundance heavy metal. Cadmium is very toxic to plants and animals, and also cause hazard to human health. It is rather mobile in soil; especially at low pH make it is easily available for plants and microorganisms. Cadmium is highly bioaccumulative as its impacts on soil microbiology were already observed at concentrations below < 1mg/ kg dm which is phytotoxic effects already at 0.35 mg/kg dm soil. Therefore, there is a high chance for mobile Cd to transfer into food plants (Vogel and Tertyze, 2006). Accumulation of Cd in kidneys with the concentration of the kidney cortex exceeds 200 mg/kg fresh weight leads to dysfunction of the kidney (Alloway, 1990).

2.1.2 Chromium (Cr)

Alloway (1990) stated that Chromium is a d-block transition metal of Group VIB with atomic number of 24 which has metal that is brittle, grey and resistant to attack by oxidation. It
enters the soil via use of phosphate fertilizer and exists as Cr (III) in soil which is not likely to be toxic. Cr (III) is quiet mobile in acid medium with its compound are very stable in soils. On the other hand, Cr (IV) is very unstable in soils and easily mobilized either in acid or alkaline soils (Kabata-Pendias and Pendias, 1989).

2.1.3 Mercury (Hg)

Daintith (2004) mercury is a heavy silvery liquid metallic element with its main ore is the sulphide cinnabar (H₂S). This element is less reactive than zinc and cadmium and thus it will not displace hydrogen from acids. On the other hand (Curtis and Smith, 2002) stated that the most common chemical species in soils are Hg⁰ and Hg²⁺ that mercury always forms. Mercury is soluble in soil water and therefore it is quite less mobile in soil.

2.2 The Soils

Soil is a thin, unconsolidated and variable layer of mineral and organic matter. It is the medium for plants growth which the roots of crops grow (Singer and Munns, 2006). Soil composed of weathered rock, minerals and decayed organic matter beside contains living materials (Ramachandra, 2006). Bohn et.al., (2001) stated that soil is a mixture of organic and inorganic solids, water, air, microorganisms and plant roots.

Soil formed through a soil formation process called “pedogenesis”. In this process, the thin layer surface of soil develops on weathered rock material which is then the thickness increase and differentiates to form a soil profile. The soil profile consists of distinct layers called horizons which are have different in colour, texture and structure (Alloway, 1990). The kind of
soil that results from the soil-forming process are controlled by five factors, which are parent material, climate, organisms, topography, and time (Singer and Munns, 2006). Major components of soils are including silicon, aluminum (Al) and iron (Fe) along with oxygen, which responsible for the soil matrix and also other components such as manganese (Mn), magnesium (Mg), potassium (K), calcium (Ca) and titanium (Ti).

Alloway (1990) stated that the hydrogen ion concentration in the solution present in soil pores is applied for the pH of a soil. It is in dynamic equilibrium with the negatively charged surfaces of the soil particles. Soil pH differs due to localized variations within the soil and usually increases with depth in humid regions and decreases with depth in arid environments. Heavy metal cations are most mobile under acid conditions and increasing the pH reduces their bioavailability.

Soils contain organic matter with the amount and type may vary considerably. The soil organic matter (SOM) has a major influence on the biological, chemical and physical properties of soils (Alloway, 1990). Their functions are to supply N, P and S for plant growth, provides energy source for soil microorganisms and promotes good oil structure (Bohn et al., 2001).

2.3 Speciation

Speciation can be defined as the different chemical form of an element which its different forms together comprise its total concentration in a given sample (Christie, 1995). On the other hand, speciation is the extraction and quantification of a soil phase which is functionally designated in that its element content. Speciation also can be defined operationally by the extraction technique itself where this techniques either single or sequential, provide information
on speciation, particle size, and the source of the metal and also quantitatively determine the metal level present.

The extraction will be designed to extract the element bound to a particular soil phase that selective extractants are use to quantify the element content in a particular phase Based on the concept pools in soils’, the elements of different solubilities and mobilities can be selectively sampled by extractants of different strength. The precise definition of speciation is the determination of precise chemical forms of oxidation states of elements which can be used for measurement of the amount of heavy metals in a soil (Alloway, 1990).

Speciation is very important as changes in speciation can affect metals toxicity. For example, the species of inorganic mercury have low bioavailability and thus low toxicity. They are generally unable to cross biological membranes. However, mercury species in alkyl form or alkyl mercury are prone to lipid soluble which is highly toxic to aquatic organisms (Christie, 1995).

Community Bureau of Reference (BCR) of the Commission of the European Communities with a group of 35 European laboratories have been carrying out a sequential extraction procedure and analysis of soils and sediments in pursuit of the quality control aspect of metal speciation (Alloway, 1990).

2.3.1 Single extraction

Single extractions procedures carried out by utilizing a separate aliquot of sample with 2 g for each individual reagent. Extracts are centrifuged and stored as in the sequential extraction schemes. The main advantage of single extraction procedure is that all fractions can be
simultaneously extracted and gives faster results than the sequential methods (L´opez et al., 2000).

However, this type of extraction procedure requires a larger sample amount. Apart from that, single extraction methods has lack of uniformity in the different procedures used and thus, the results obtained are operationally defined depending on the experimental conditions used (type and concentration of extracting agent, soil mass: volume ratio, shaking time and speed of shaking). In consequence, the data comparison would be difficult beside it would prevent the standardisation of these methods (López-Sánchez et al., 2003).

Meers et al., (2006) mentioned that the extractability of Cd, Cr, Cu, Ni, Pb, and Zn in a dredged sediment disposal site for the studies of availability of heavy metals for uptake by Salix viminalis on a moderately contaminated dredged sediment disposal site was extracted using single extraction procedures (H$_2$O; 0.01 M CaCl$_2$; 1 M NH$_4$OAc; NH$_4$OAc-EDTA; CaCl$_2$-TEA-DTPA). The results for this study showed that only Cd and Zn were found to exceed statutory threshold values for total content.

The study of the copper distribution in contaminated soils of hop fields single extraction procedures were carried out with EDTA, acetic acid, deionised water, ammonium nitrate and calcium chloride were used as the extractants. In this study the extraction behaviour of copper was determined from the contaminated and non-contaminated soils. The study indicates that there was no vertical copper translocation in the ground was observed (Schramel et al., 2000).
2.3.2 Sequential extraction

Sequential extraction is one of the popular methods for heavy metals chemical speciation (Tokalio˘glu, 2001). In order to determine the availability of metals, sequential extraction procedures can be used to provide and estimate the different associations of heavy metals with the various soil components (Ahumada, 2006). There are many factors affect the success in selective extraction using sequential extraction. Among of them are the chemical properties of the extractant choosen, its extraction efficiency, experimental parameters, the sequence of the individual steps,

The sequential extractions have been applied using extractants with increasing extraction capacity (Tokalio˘glu, 2001). Sequential extraction schemes have been developed to predict the metal distribution among different fractions including metal exchangeable, associated to carbonates, associated to Fe–Mn oxides (or reducible), associated to organic matter and sulfides (or oxidizable) and residual. These techniques are useful in the study of metal uptake by plants and soil invertebrates where transfer takes place from a water solution phase (L´opez et al., 2000). For heavy metals extracted by using sequential extraction procedure, soil or sediments sample is applied with various types of chemical extractants in which each follow-up treatment being more drastic in chemical action or of a different nature than the previous one.

There are many sequential extraction procedures can be found in the literature since the 1970s (L´opez et al., 2000). Vicente-Beckett et al., (2003) stated that the first scheme for metal speciation analysis was reported by Tessier et al., (1979). The proposed scheme contains a five steps extraction scheme which divides the total metal content into five reactions, which are the exchangeable, carbonate bound, iron/manganese oxide bound, organic bound and residual
fraction (Tokalioğlu, 2001). The scheme developed by Tessier et al is for sediments as well as for soils (Abollino et al., 2002).

Then the Standards, Measurements and Testing programme (SM & T) formerly known as Community Bureau of Reference (BCR) of the European Union has proposed a three-stage sequential extraction procedure for the determination of metals such as Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in soil samples (Tokalioğlu, 2001). This most recent sequential extraction method has been developed to harmonize different methodologies of the extraction process (L´opez et al., 2000) and it is a recommended standard procedure for the characterization of heavy metals in soils and sediments (Davidson et al., 1995).

Sequential extraction provides some advantages which are hardly to be found in single extraction. Kashem et al., (2007) mentioned that the sequential extraction procedures define the fractions of elements in soil more precise than single extraction. Besides, Cuong and Obbard (2006) stated that heavy metal speciation in environmental media using sequential extraction techniques offers a more realistic estimate of actual environmental impact. However, sequential extraction consumes more time than single extraction and requires skilled personal and sufficient analytical facilities (Kashem et al., 2007).

2.4 Metals extraction in soils

To evaluate metal availability in soils many extraction procedures exist. They can be divided in two groups which are sequential extraction and simple or single extraction (Marín et al., 2001). Metals extraction in soil for heavy metals speciation studies carried out by single or sequential extraction with the reagents having different chemical properties (Abollino et al.,
2002). Many single or sequential extraction procedures have been applied to soils and sediments to fractionate metals to obtain more useful information about the bioavailability and mobility of metals (Tokalio˘glu, 2001).

Soil sequential extraction is performed according to the procedure proposed by the Community Bureau of Reference (BCR). The proposed procedures consider three extractions to obtain an acid-soluble fraction (exchangeable, carbonates), a reducible fraction (iron/manganese oxides), an oxidizable fraction (organic substance and sulphides) (Ahumada et al., 2006).

There are various extraction media that have been widely used to assess the reactivity of binding forms of heavy metals in solid materials such as in soils or sediments. Among them are electrolytes (CaCl₂ or MgCl₂), pH buffers of weak acids (acetic or oxalic acid), chelating agents (EDTA or DTPA), reducing agents (NH₂OH), strong acids (HCl, HNO₃, HClO₄, HF) or basic reagents (NaOH, Na₂CO₃. (Tokalio˘glu, 2001).

Batley (1991) stated that high proportions of Cd, Zn, Pb, and Ni were found in soluble and/or exchangeable forms in the study of metals in urban soil. This suggests that the elements may have immediate impact on the quality of receiving waters and can be easily available for organisms in soil. Meanwhile, according to the study of six street dust and four roadside soils by Harrison et al., (1973), Cd was present to an appreciable extent in the exchangeable fraction while Pb was present in acid-soluble fraction in which they bound to carbonates and Fe/Mn oxyhydrates. The total metal concentrations of these metals suggested that the order of contamination of Pb is more than Cd. Pb is primarily associated with other than ion-exchangeable forms and would be highly immobile and difficult to leach down the soil profile.
Thus, Pb was expected to be taken up only to a minor extent by plant roots and soil biota (Batley, 1991).

Abollino et al., (2002) reported that the heavy metals speciation in five agricultural soils of Piedmont Region in north-western Italy was carried out by applying Tessier’s sequential extraction procedure. He stated that among the five soils, the soil from Maurizio Canavese has the highest concentration of Cr and Ni, probably due to the nature of the parent material, which consists of ultramafic rocks. A high Pb concentration was found in Vigone soils which could be derived from the parent material or to atmospheric deposition deriving from a distant source. The concentrations of the metals in the fraction bound to organic matter and to sulphides are lower than in the fraction bound to iron and manganese oxides.

Shan (2003) had conducted a single microwave extraction procedure to investigate the bioavailability of rare earth elements (REEs) by using different extractants of 0.05 M EDTA, 0.10 M CH₃COOH, 0.10 M HCl, and 0.05 M CaCl₂. The study showed that the extraction efficiency varied with different type of extractant. EDTA yielded higher extractant efficiency than HCl but HCl yielded much higher extractant efficiency than CaCl₂ and CH₃COOH.

According to Cuong and Obbard (2006), the speciation of Cd, Cr, Cu, Ni, Pb and Zn heavy metals in marine sediments from two coastal regions of Singapore (Kranji and Pulau Tekong) was determined using the 3-step sequential extraction procedure developed by the European Community Bureau of Reference (1999). The study reported that all metals except for Cd were present at higher percentages in the acid-soluble fractions (the most labile fraction) in Kranji relative to those in Pulau Tekong. Heavy metal Cd was present at the highest level in the acid-soluble fraction meanwhile Cr was present at the highest level in residual fraction. The
highest level of Cu was found in the oxidizable fraction indicating that they were mainly bound to organic matter and sulfides meanwhile Ni and Pb were found in the residual fractions in both Kranji and Pulau Tekong.
CHAPTER THREE

MATERIAL AND METHODS

3.1 Sampling Location and Collection

Peat soils located in pineapple farm in the area of pineapple development project in Kampung Meranek, Kota Samarahan (Figure 3.1) were chosen for this study. The pineapple farm was selected because it represents an agricultural area in which various kinds of fertilizers were applied in the soil. NPK fertilizer (N: P₂O₅: K₂O ratio is 15: 15: 15), Behn Meyer, Supreme Green and ABF Granule Urea (12-12-17-2+ Trace Elements) were among fertilizers applied in the soil.

![Sampling Location Diagram]

* F= soils with fertilizer application  
* NF=soils without fertilizer application

Figure 3.1: Sampling location at the pineapple farm in Kampung Meranek, Kota Samarahan (Source: Land and Survey Department of Kota Samarahan, 2007).