Faculty of Resource Science and Technology

# DISTRIBUTION OF HEAVY METALS IN SOIL AND TWO PLANT SPECIES (*ASYSTASIA COROMANDELIANA* AND *PHYLLANTHUS NIRURI*)

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## (*ASYSTASIA COROMANDELIANA* AND *PHYLLANTHUS NIRURI*)

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## DECLARATION

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

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# ACKNOWLEDGMENTS

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# Distribution of Heavy Metals in Soil and Two Plant Species

## (*Asystasia coromandeliana* and *Phyllanthus Niruri*)

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## ABSTRACT

The pollution of the natural environment is and will remain forever, one of the important problems for the mankind. Two plant species namely *Asystasia coromandeliana* and *Phyllanthus niruri* were used to show the uptake of heavy metals from soil and distribution of heavy metals in their tissues samples. The analysis of Cd, Cr and Cu were carried out by using atomic absorption spectrophotometer (AAS). *P. niruri* had absorbed concentration of Cr and Cu in soil efficiently according to comparison between distribution of Cr and Cu in its root, stem and leaf with concentration of the heavy metals in soil. The same scale showed by *A. coromandeliana*, where the concentration of Cr and Cu uptake by its tissues samples was much higher and consistent than extraction of Cd. The high amounts of Cu and Cr distributed in tissues samples for both plant species were actually related to their potentials to uptake the heavy metals. Both of plants also showed the distribution of heavy metals was higher in root, followed by stem and leaf. The absorption of plants showed that *P. niruri* absorbed more Cr (103.66 mg/kg), followed by Cu (12.66 mg/kg) and Cd (12.60 mg/kg), whereas *A. coromandeliana* absorbed more Cu (112.28 mg/kg) and quite high of Cr (104.74 mg/kg), but absorbed only 4.9 mg/kg of Cd. The distribution pattern of heavy metals in these two plant species indicates that both of the plants are suitable as accumulator to absorb heavy metals from soil for remediation purposes.

**Keywords:** Heavy metals, *Asystasia coromandeliana, Phyllanthus niruri*

# ABSTRAK

*Pencemaran terhadap persekitaran semulajadi sentiasa dan akan kekal untuk selamanya, dan ini merupakan salah satu masalah penting kepada hidupan. Dua spesies tumbuhan iaitu Asystasia coromandeliana dan Phyllanthus niruri telah digunakan untuk menunjukkan tindakkan penarikan logam-logam berat daripada tanah dan taburan logam-logam berat di dalam sampel-sampel tisu. Analisis Cd, Cr dan Cu dilakukan menggunakan spektrofotometer serapan atom (SSA). P. niruri menyerap kepekatan Cr dan Cu dalam tanah dengan berkesan berdasarkan kepada perbandingan antara taburan Cr dan Cu dalam akar, batang dan daunnya dengan kepekatan logam berat tersebut yang terdapat dalam tanah. Skala yang sama ditunjukkan oleh A. coromandeliana, di mana kepekatan Cr dan Cu diserap oleh tisu-tisu sampelnya adalah lebih tinggi dan konsisten berbanding pengekstrakan Cd. Kuantiti yang tinggi oleh Cu dan Cr yang ditaburkan di dalam sampel-sampel tisu untuk kedua-dua spesies tumbuhan bergantung kepada keupayaan tumbuhan-tumbuhan tersebut untuk menarik logam-logam berat. Kedua-dua tumbuhan juga menunjukkan taburan logam berat lebih tinggi di dalam akar, diikuti dengan batang dan daun. Penyerapan oleh tumbuhan menunjukkan bahawa P. niruri menyerap lebih banyak Cr (103.66 mg/kg), diikuti oleh Cu (12.66 mg/kg) dan Cd (12.60 mg/kg), manakala A. coromandeliana menyerap lebih banyak Cu (112.28 mg/kg) dan agak tinggi kepekatan Cr (104.74 mg/kg), tetapi menyerap hanya 4.9 mg/kg kepekatan Cd. Corak taburan logam berat dalam dua spesies tumbuhan ini menunjukkan bahawa kedua-dua tumbuhan tersebut sesuai digunakan sebagai bahan penumpuk untuk menyerap logam berat daripada tanah untuk tujuan pemuliharaan.*

*Kata kunci:* *Logam berat, Asystasia coromandeliana, Phyllanthus niruri*

# CHAPTER ONE

# GENERAL INTRODUCTION

## 1.1 Introduction

The enrichment of agricultural land with potentially toxic metals can be damaging to soil health and may constitute a risk to human health resulting from consumption of food crops. Agricultural land may be contaminated by potentially toxic metals from a variety of sources including anthropogenic inputs such as the application of sewage sludge (Maxted et al., 2007; Sinha et al., 2007). In most of the plants, exposure to elevated concentration of heavy metals resulted in growth inhibition. After prolonged metal exposures, sensitive plants develop visible symptoms of toxicity such as chlorosis, and necrotic lesions. Heavy metals can bind to functionally important domains of biomolecules and thereby inactive them. The result can be, for instance, the inhibition of an enzymatic reaction, and disturbance of metabolism (Sinha et al., 2007). In plants, changes in membrane permeability lead to water-stress like conditions, which results in an increase in proline levels. Proline accumulation is accepted as an indicator of environmental stress including exposure to heavy metals and also considered to have important protective roles (Sinha et al., 2005).

The influence of heavy metals in contaminated soils on concentrations of these elements in plants has been widely studied and it is generally accepted that total metal concentrations in soil do not provide a good indication of the levels potentially available to plants (Ullrich et al., 1999). Plants may be passive receptors of trace elements (fallout interception or root adsorption), but they also exert control over uptake or rejection of some elements by appropriate physiological reactions. Special attention also should be given to the

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forms of metals distributed within plant tissues, for the metal forms in plants seem to have a decisive role in metal transfer to other organism (Kabata pendias and Pendias, 1989). Toxic metals, such as Cd and Cr are still increasingly accumulating in urban environment, causing concern because urban soil have a direct influence on public health ast hey easily come in contact with humans (Salonen and Korkka-Niemi, 2007).

Around the world several studies have evaluated the heavy metal concentrations in soils and distribution in plants (Kabata-Pendias and Pendias, 1989). Numerous efforts have been made to develop technologies for the remediation of contaminated soils, including ex situ washing with physical-chemical methods and the in situ immobilization of metal pollutants. However, these methods of clean up are generally very costly and often harmful to properties of soil (Chen et al., 2004).

In this study, two different plant species belonging to the *Euphorbiaceae* family (*Phyllanthus niruri*) and *Acanthaceae* family (*Asystasia coromandeliana*) have been studied for distribution of heavy metals and uptake the metals by plants. In order to clarify the spatial distribution of heavy metals and extent of contamination in the sampling area, this follow-up investigation was carried out.

## 1.2    Objectives of the Project

The purpose of this study was to determine the types of heavy metals that contains in soil where sampling area at greenhouse in East Campus of UNIMAS, specifically in determining copper (Cu), cadmium (Cd) and chromium (Cr). These heavy metals were chosen because according to the previous research, the concentration of these heavy metals were obviously more higher and commonly present compared to other trace element that contain in soil. This study also were to assess the distribution of heavy metals in three different parts of plants (root, stem and leaf) and in other word was to determine the potential of selected plants as the bioaccumulator species, and the uptake of heavy metals by two selected plant species.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1    Heavy Metal

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cation, etc.). The most heavy metal contaminants are Cd, Cr, Cu, Pb and Zn. Metals are natural components in soil (Lasat, 2000). Heavy metals, in particular, originate from industrial emissions, mining activity, disposal of wastes and fertilizer and pesticide use (Marchiol et al., 2004). A large number of sites worldwide are contaminated by heavy metals as a result of human activities (Lombi et al., 2001).

### 2.1.1    Heavy Metals in Soil and the Effect of Soil pH

There is possibility to find many types of heavy metals in soil and sludge. Maxted et al. (2007) reported that the uptake of heavy metals generally increased with decreasing soil pH and the uptake of Cd and Zn are highest at pH value between 5 to 6 depending on the soil metal loading. As a first step to determine the reference levels of heavy metals, it is necessary to know their contents in soils under natural conditions (Navas and Machin, 2002).

Dwiecki and Koziol (2005) have conducted a phytoextraction study on five of heavy metals exist in various part of *Reynoutria japonica* namely iron (Fe), cadmium (Cd), lead (Pb) zinc (Zn) and mercury (Hg). The results from this study are shown in Table 2.1. It was found that zinc (Zn) is a major heavy metal present in soil.

**Table 2.1 :** Contents of heavy metals in parts of *Reynoutria japonica* (in mg/kg of dry   mass) (Dwiecki and Koziol, 2005).

| Well grown plants | Fe | Cd | Pb | Zn | Hg |
|---|---|---|---|---|---|
| Main stems | 7.8 | 0.03 | 0.57 | 10.1 | 9.2 |
| Side stems | 0.0 | 0.06 | 0.0 | 7.3 | 0.0114 |
| Leaves | 43.5 | 0.16 | 1.2 | 13.2 | 0.0168 |
| Flowers | 10.3 | 0.12 | 1.4 | 14.5 | 0.0148 |

Lombi et al. (2001) reported that Cd and Zn can be accumulated by *Thlaspi caerulescens* (the Ganges ecotype from southern France). The affection of metal containment is particularly true in the case of Zn where leachability increased in composted sewage sludge. The increased leachability of Zn from sewage sludge amended soil could be caused by the formation of complexes of Zn with organic matter compounds from the composted sewage sludge, such as humic and fulvic acids or by changes in pH with the addition of sewage sludge (Herwijnen et al., 2007). They also stated that the effect of compost amendment depends on the type of compost, the soil type and contaminant levels.

### 2.1.2   Heavy Metals Effects in Health

The accumulation of heavy metals in environmental samples represents a potential risk to human health due to the transfer of these elements to aquatic media, their uptake by plants and their subsequent introduction into the food chain. The toxicity, bioavailability and mobility of metals are related to their species (Erika-Andrea et al., 2005).

## 2.2    Soils Contaminant

Soil contamination is the presence of man-made chemicals or other alteration of the natural soil environment. Metals are natural components in soil. Contamination, however, has resulted from industrial activities such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, and generation of municipal waste (Lasat, 2000). Soils are prone to contamination from atmospheric and hydrological sources, but direct waste disposal causes a major impact on this natural resource, posing serious environmental concerns (Navas and Machin, 2002). Regardless of their origins and the reasons for the increase in their concentration in soils, trace metals are liable to contaminate food chains by migrating toward groundwater or by accumulating in plants (Selim and Sparks, 2001). The influence of heavy metals in contaminated soils on concentration of these elements in plants has been widely studied and it is generally accepted that total metal concentrations in soil do not provide a good indication of the levels potentially available to plants (Ullrich et al., 1999).

## 2.3    Phytoextraction

Remediation methods for heavy metal removal from soil and sludge are expensive and disruptive. Recently, efforts have been directed toward finding remediation strategies that are less expensive and less damaging to soil properties than current approaches. One such method is phytoextraction where plants species are used to uptake heavy metals from the soil or sludge (Pogrzeba et al., 2001)

### 2.3.1    Uptake of Heavy Metals by Plants

The significant role of plants in both cycling of trace elements and contaminating the food chains has been well illustrated for various ecosystems and published in numerous papers (Kabata-Pendias and Pendias, 1989). Plants can accumulate trace elements, especially heavy metals, in or on their tissues due to their great ability to adapt to variable chemical properties of the environment. Thus plants are intermediate reservoirs through which trace elements from soils, and partly from waters and air, migrated to man and animals. Several plants may be passive receptors of trace elements but they also exert control over uptake or rejection of some elements by appropriate physiological reactions (Kabata-Pendias and Pendias, 1989). A schematic representation of metal transport processes that take place in roots and shoots is shown in Figure 2.1.
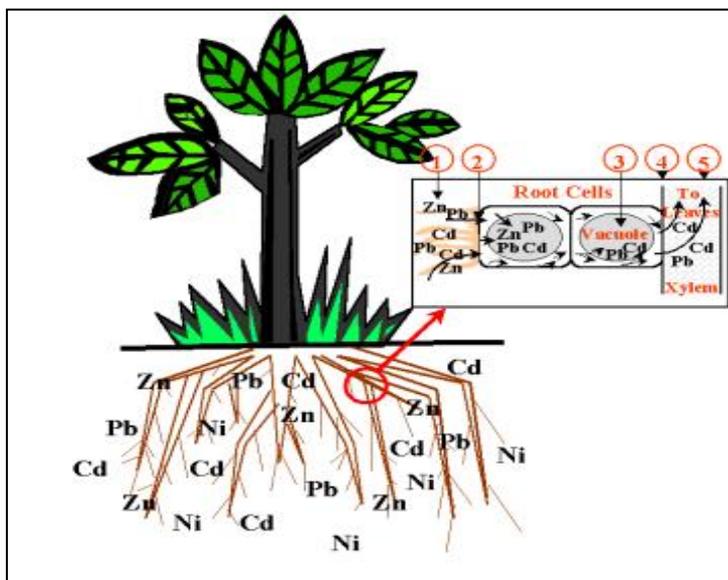


1. A metal fraction is sorbed at root surface.
2. Bioavailable metal moves across cellular membrane into root cells.
3. A fraction of the metal absorbed into roots is immobilized in the vacuole.
4. Intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem).
5. Metal is translocated from the root to aerial tissues (stems and leaves).

**Figure 2.1 :** Metal uptake and accumulation in plants (Lasat, 2000).

To grow and complete the life cycle, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo. Plants have evolved highly specific mechanisms to take up, translocate, and store these nutrients. For example, metal movement across biological membranes is mediated by proteins with transport functions. In addition, sensitive mechanisms maintain intracellular concentration of metal ions within the physiological range. In general, the uptake mechanism is selective, plants preferentially acquiring some ions over others. Ion uptake selectivity depends upon the structure and properties of membrane transporters. These characteristics allow transporters to recognize, bind, and mediate the transmembrane transport of specific ions. For example, some transporters mediate the transport of divalent cations but do not recognize mono- or trivalent ions (Lasat, 2000).

The development of large-scale phytoextraction techniques could consider plants species as bioaccumulators of heavy metals. These plants can accumulate heavy metals while producing high biomass in response to established agricultural management (Marchiol et al., 2004). This approach has been carried out using maize (Pogrzeba at al., 2001; Lombi et al., 2001), ryegrass of *Lolium perenne L.* (Herwijnen et al., 2007), sunflower (Chen et al., 2004) and fast growing tress such as *Thlaspi caerulescens* or *Salix* spp. (Nevel et al., 2007; Maxted et al., 2007) as bioaccumulator of heavy metals have also been studied.

### 2.3.2 Distribution of Heavy Metals in Different Parts of Plant

Concentrations of heavy metals are higher in root than upper parts of plant (Sinha et al., 2007). This can be shown in the tables below where previous studies by Porgzeba et al. (2001), Marchiol et al. (2004) and Sinha et al. (2007) reported that distribution of heavy metals in plants were higher in roots than in shoots.

**Table 2.2 :** Concentration of Cd in parts of plants (mg/kg dry mass)

| Parts of plant | Other studies | |
| --- | --- | --- |
| | white mustard (Pogrzeba et al., 2001) | *B. napus* (Marchiol et al., 2004) |
| Root | 3.75 | 0.91 |
| Shoot | 1.26 | 0.61 |

**Table 2.3 :** Concentration of Cr in parts of plants (mg/kg dry mass)

| Parts of plant | Other studies | |
| --- | --- | --- |
| | fenugreek plants  (Sinha et al., 2007) | *B. napus* (Marchiol et al., 2004) |
| Root | 117.24 | 5.77 |
| Shoot | 90.21 | 0.76 |

### 2.3.3   Plants Tolerate High Metals Concentration in Soil

Ecological studies have revealed the existence of specific plant communities, endemic floras, which have adapted on soils contaminated with elevated levels of Zn, Cu, and Ni. Different ecotypes of the same species may occur in areas uncontaminated by metals. To plants endemic to metal-contaminated soils, metal tolerance is an indispensable property. In comparison, in related populations inhabiting uncontaminated areas, a continuous gradation between ecotypes with high and low tolerance usually occurs (Lasat, 2000).

Plants evolved several effective mechanisms for tolerating high concentrations of metals in soil. In some species, tolerance is achieved by preventing toxic metals uptake into root cells. These plants, coined excluders, have little potential for metal extraction. Such an

excluder is "Merlin," a commercial variety of red fescue (*Festuca rubra*), used to stabilize erosion-susceptible metal-contaminated soils. A second group of plants, accumulators, does not prevent metals from entering the root. Accumulator species have evolved specific mechanisms for detoxifying high metal levels accumulated in the cells. These mechanisms allow bioaccumulation of extremely high concentration of metals. In addition, a third group of plants, termed indicators, show poor control over metal uptake and transport processes. In these plants, the extent of metal accumulation reflects metal concentration in the rhizospheric soil (Lasat, 2000).

## 2.4     Weed Species in Malaysian Agro-Ecosystems

*Asystasia coromandeliana* and *Phyllanthus niruri* are including in the weeds family. The warm tropical climate of Malaysia with adequate rainfall and available nutrients permits the luxuriant growth of crops and weeds alike almost all year round. This, coupled with mass transport of goods and the populace, continuous opening and exploitation of new farming areas, intensive agricultural and forestry activities, urbanization, abandoned and derelict farmlands, and fragmentation of natural habitats and agropastoral sites, among others, are some of the driving forces that increase the movement of weed species across natural boundaries within the country (Baki, 2004).

The terrestrial invasives include the wide spread of *Imperata cylindrica, Ischaemum rugosum,* the *Echinochloa* species aggregates*, Pennisetum polystachion, Fimbristylis milicea, Cyperus rotundus, Scleria sumatrensis, Scirpus grossus, Eleusine indica, Leptochloa*

*chinensis, Melastoma malabathricum, Mikania micrantha, Pueraria phaseoloides, Calopogonium cereleum, Chromolaena odorata, Mimosa pudica, Mimosa invisa, M. pigra, M. quadrivalvis,* and *Asystasia gangetica* in many agricultural areas, along roadsides, railway tracks, and in derelict and abandoned sites (Baki, 2004).

# CHAPTER THREE
# MATERIALS AND METHODS

## 3.1    Plant and Soil Samples

Two species of plants native to Sarawak have been used to investigate the distribution of heavy metals in plants. The plant species were *Asystasia coromandeliana* and *Phyllanthus niruri.* The details of these species are presented in Table 3.1.

**Table 3.1 :** Plant species selected for distribution of heavy metals study.

| Name of species | Comman names | Local  names |
|---|---|---|
| *Asystasia coromandeliana* | Coromandel | - |
| *Phyllanthus niruri* | Seed on the leaf / pick-a-back | Dukung anak |

Soil and plant samples were collected from a greenhouse area at East Campus of Universiti Malaysia Sarawak. The roots, stems and leaves of plants were analyzed for heavy metal contents together with soil where it grown. The fresh samples were immediately transported to the laboratory for treatment. The soil samples were air dried and sieved to soil lump sizes smaller than 2.0 mm. While the shoots were sampled by cutting off all shoots at 1 cm above the soil; roots were sampled by separated them from the soil mixtures and washed them three times with deionized water (Herwijnen et al., 2007). Then, the plant parts were oven dried to remove water at 80℃ for 24 hours. The dried plant parts were reduced 1.0 to 1.5 mm particle size by cutting and grinding.

## 3.2 Sample Preparation of Soils and Plants

### 3.2.1 Soil Samples Preparation

The dried soil samples were weighed 1g into a beaker. 10ml 1:1 $HNO_3$ were added, the slurry were mixed and covered with watch glass. Then, reflux for 10 to 15 minutes. After cooled, 5ml concentrated $HNO_3$ were added, replaced the cover and refluxed for 30 minutes. These steps were repeated until digestion complete by added 5ml of concentrated $HNO_3$ over and over until no brown fumes given off by the samples and samples were evaporated to approximately 5ml and cooled. The volume was reduced approximately to 5ml and cooled (Kimbrough and Wakakuwa, 1989). Lastly, samples were filtered by using Whatman No. 41 filter paper and marked up to volume.

### 3.2.2 Plant Samples Preparation

Plant samples (1.0 g each) were taken at random from the composite sample and were processed for analysis by the dry-ashing method (Kalra, 1998). 1 g of dried ($80^0C$) and grounded plant material were placed in a porcelain crucible. The samples were placed in a cool muffle furnace. It was dried in the furnace at $500^0C$ and then left to ash at these temperatures for 4 to 8 hours (Crosby, 1997). The sample were left to cool in the extent of destruction were checked. Then, the tissues were moisture with $HNO_3$ to cover ash. Repeat this step, if necessary, until a clean white ash is obtained. Next, the $HNO_3$ were evaporated from the sample on a hot plate. The step will be repeated again. The samples were solubilized using diluted $HNO_3$ and brought into final solution with deionized water in volumetric flask. Finally, the ash suspensions were filtered into volumetric flask using Whatman filter paper No.41.