

ACCUMULATION OF HEAVY METALS AND PHYSIOLOGICAL CHANGES OF *Melaleuca cajuputi* PLANTED IN CONTAMINATED SOIL.

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1. Introduction

Soil pollution has been a major environmental issue over the years. It is mainly occurs due to human activities such as dumping waste, agricultural practices, deforestation and mining. Heavy metal instigate by two primary sources which is natural inputs and anthropogenic inputs (Zhang, 2006; Chen, 2008). In order to perform any pollution remediation, Chen (2008) points out that, it is very necessary to identify metal source. Agriculture waste can be either crop residues or livestock manure. Bioremediation has been a popular means of treating pollution in these few decades. The term bioremediation is used by Suresh *et al.* (2004) to refer to the used of biological agents for bioremediation of soils and solutions. One type of bioremediation that is widely known is phytoremediation. A variety of definitions of the term phytoremediation have been suggested, this study will used the definition suggested by Alkorta (2004) who saw it as an emerging cost effective, non-intrusive, and aesthetically pleasing technology, that used ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues, appear very promising for the removal of pollutants from the environment. Plant physiology is defined as the study of natural phenomena in living plants. It is the science concerned with processes and functions, the response of plants to change in the environment, and the growth and development which result from responses (Noggle, 1976; Mohr et al., 1995). Physiological changes may occur due to inhibitory effects of heavy metals on photosynthesis, transpiration, carbohydrate metabolism and other metabolic activities.

In this study, *Melaleuca cajuputi* will be used to treat contaminated soil. The leaves of *Melaleuca cajuputi* possess antibacterial, anti-inflammatory and anodyne properties and are used traditionally against pain, burns, colds, influenza and dyspepsia. *Melaleuca cajuputi* is a well known species that produce cajeput oil. The oil was found to contain substantial amounts of 1,8-cineole, sesquiterpene alcohols globulol, viridiflorol and spathulenol. Plants consist of secondary metabolite and primary metabolites. Secondary metabolites can be divided into three major classes that are phenolics, alkaloids and terpenoids. Primary metabolites include carbohydrates, proteins and lipids. Secondary metabolite that will be studied in this research is phenolics. Phenolics are compounds which possess an aromatic ring bearing a hydroxyl group or its substituent. It is a very effective antioxidant. The literature review part begins by laying out the theoretical dimensions of the research and looks at physiological changes due to toxicity studies done by others. Research methodology describes the design, synthesis, characterization and evaluation of growth parameters, biochemical parameters and heavy metal analysis.

2. Problem statement and significance of study

People have been using medicinal plants as alternative medicine. But, the problem is whether medicinal plants really safe to be consume. Medicinal herbs may be easily contaminated during growing and processing. We need to grow medicinal plants on heavy metal polluted soil without affecting the end marketable product. Recent reviews of the scientific literature on herbal medicinal products have a warning that they are not free if risk. Phytoremediation can be an option for mildly heavy metal polluted soil.

3. Research objective

The purpose of this study is to:

1. determine the accumulation of heavy metal in the roots, leaves and stems of the plant soils.
2. study the effect of heavy metal on photosynthetic parameters, growth parameters, and biochemical parameters.

4. Literature review

Itoh (2002) highlight that, there has been a mounting amount of industrial and municipal waste is discharge in the manufacturing process and after public or household used. Soils may be polluted by a wide range of contaminants from industrial activities, sewage sludge disposal, metal processing and energy production (Lim, 2004). Heavy metals instigate by two primary sources which is natural inputs and anthropogenic inputs (Zhang, 2006; Chen 2008). Example of natural input is parent material weathering. Metalliferous industries and mining, vehicle exhaust and agronomic practices may contribute to antropogenic inputs. Chen (2008) points out that, it is very necessary to identify metal source before performing any pollution remediation. Bioremediation has received a lot of attention by researchers (Suresh *et al.*, 2004). The scientific basis for bioremediation is the manipulation of living systems to effect desired changes in the environment (Sriprang, 2007) .Bioremediation is divided into two main groups as states by Bruschi, (2007) that is direct reduction by enzymatic metal reductase activity of the bacteria and indirect reduction by the use of hydrogen sulphide, biologically produced to reduce and precipitate metals. There are various techniques of bioremediation. The process of bioremediation using plants is known as phytoremediation. Phytoremediation as suggest by Zhu and Shaw (2000) is an umbrella term which covers several plant-based approaches for cleaning up contaminants. A study done by Bharagava (2008) revealed that a plant species are well adopted to tolerate and accumulate high quantities of trace elements due to increase level of antioxidant.

5. Research methodology

5.1 Plant material and growth condition

Seedlings of *Melaleuca cajuputi* will be grown under control condition in the greenhouse. The seedlings will be planted in contaminated and control soil. Industrial sewage sludge will be used as treatment. The sludge will be screened for heavy metal content to identify the element to be trace inside the plant sample using ICP.

5.2 Experimental design

Randomized complete block design (RCBD) with 4 blocks (months), 6 treatments (level of sludge) and six replications.

M1	S1	S5	S6	S2	S5	S3	S4	S5	S2	S6	S5	S3	S1	S2	S6	S5	S3	S4
	S2	S3	S4	S6	S4	S1	S6	S1	S4	S2	S4	S1	S4	S5	S3	S1	S2	S6
M2	S2	S1	S3	S5	S6	S4	S3	S2	S1	S5	S4	S6	S3	S1	S2	S4	S6	S5
	S4	S6	S5	S2	S1	S3	S5	S6	S4	S3	S2	S1	S5	S4	S6	S3	S1	S2
M3	S5	S4	S6	S1	S3	S6	S6	S2	S3	S2	S1	S4	S6	S2	S1	S3	S5	S4
	S1	S3	S2	S5	S4	S2	S4	S1	S5	S5	S6	S3	S3	S5	S4	S1	S6	S2
M4	S1	S2	S6	S3	S5	S6	S6	S1	S5	S2	S4	S3	S5	S3	S4	S2	S1	S6
	S4	S3	S5	S2	S1	S4	S2	S4	S3	S5	S6	S1	S1	S6	S2	S3	S4	S5

5.3 Sampling and analysis

5.3.1 Photosynthetic parameters

Net photosynthesis rate will be measured weekly by using a portable photosynthesis equipment. This method will be done to find the relationship between photosynthetic rate and stomatal conductance to water vapor. With this, effect of heavy metals on photosynthesis rate can be determined.

5.3.2 Growth parameters

After 30, 60, 90 and 120 days of sowing, plant will be harvested. The major parts of the plant specifically the roots, stems and leaves will be separated. Growth parameter such as root length, shoot length, leaf number, height, number of leaves per seedling will be measured.

5.3.3 Biochemical parameters

Chlorophyll content of the fresh leaves sample will be estimated using Arnon's method by using spectrophotometer (Arnon, 1949). In order to determine whether there is any changes in protein content, protein content of shoots, roots and leaves of treatment and control soil will be estimated by using BSA as standard protein by using Lowry's method (Lowry, 1951). As for lipid peroxidation, it will be determined indirectly by MDA content by thiobarbituric acid (TBA) (Heath and Parker, 1968). The total content of phenolic will be assayed spectrophotometrically using method describe by Grubestic *et al.* (2005). Individual phenolic compound in the sample will be analyzed using high performance liquid chromatography (HPLC) following procedures describe by Marquez-Garcia *et al.* (2009). The antioxidant activity of the total phenolic compound will be determined using nitric oxide method while the isolated phenolics will be determined by the ferric thiocyanate method as describe by Nagatsu *et al.* (2004) and modified by Demirbas (2009).

5.3.4 Metal analysis

Element that were found in the sludge will be focused in plant analysis using inductively coupled plasma atomic emission (ICP) spectrometry as describe by Severo (2004) and modified by Barbosa *et al.*, (2007).

5.3 Statistical analysis

Differences between metal polluted soils and plants responses will be test for significance using ANOVA and separation of means using LSD Test ($p=0.05$).

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