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EVALUATION OF HEAVY METAL UPTAKE BY SENDUDUK (Melastoma malabathricum) AS A PHYTHOREMEDIATOR OF SOIL CONTAMINATED WITH SEWAGE SLUDGE

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By

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DEDICATION

Dedicated to my beloved family and the people who are special to me for supporting and inspiring me during my entire study period

ABSTRACT

Phytoremediation is the use of plants to remediate pollutants from soils and this approach has not been widely practiced in Malaysia. This study was conducted in a greenhouse, at the Faculty of Forestry, Universiti Putra Malaysia. The objectives of this study were: (i) to examine the ability of *Melastoma malabathricum* for taking up heavy metals from soil contaminated with sewage sludge in the growth medium after harvesting period and (ii) to evaluate the potential of *M. malabathricum* as a phytoremediator plant in taking up heavy metals and translocate heavy metal elements in plant parts (leaves, stems, roots) by using an indicator of Translocation factor (TF) and Bio-concentration factor (BCF). *M. malabathricum* were germinated at the greenhouse for two months prior transplanting into growth media (soil + sewage sludge) for six months. The seedlings were planted on six different growth media (soil + different levels of sewage sludge) namely; control T_0-Control (100% soil), T_1 (80% soil and 20% of sewage sludge), T_2 (60% soil and 40% of sewage sludge), T_3 (40% soil and 60% of sewage sludge), T_4 (20% soil and 80% sewage sludge) and T_5 (100% sewage sludge). Each type of treatments was replicated for four times. pH both of growth media was determined using galss electrode whereas the concentrations of heavy metals consist of copper (Cu), lead (Pb) and Zinc (Zn) in the growth media were analyzed using atomic absorption spectrometer (AAS). The pH increased after harvesting (5.39 to 6.43), compared to before planting (5.17 to 6.13). The highest value is T_5 (6.43), followed by T_4 (6.31) and the lowest T_0 (5.39). *M. malabathricum* are able to uptake heavy metals (Cu, Pb and Zn). The results showed that there the concentrations of heavy metals among treatments before planting and after harvesting were significant differences whereby before planting the highest concentration of Cu was detected at T_5 (3.93 mg kg^{-1}) followed by T_4 (2.90 mg kg^{-1}), T_3 (2.34 mg kg^{-1}) and the lowest is T_0 (0.91 mg kg^{-1}), whereas after harvesting, the Cu concentrations decreased in all treatments. The highest decreased was in T_5 (1.50 mg/kg) and the lowest is T_1 (0.26 mg kg^{-1}). The highest concentration of Pb was found in T_5 (9.54 mg kg^{-1}) and the lowest is T_0 (0.93 mg kg^{-1}) before planting. After planting, the reduction was 0.26 mg kg^{-1} for T_0 and 6.30 mg kg^{-1} for T_5. For Zn, *M. malabathricum* was very effective in removing the heavy metal in T_5 which is at the level of reduction was 14.28 mg kg^{-1}. The highest accumulation of Cu in the plant parts was in root of T_0 (0.13 mg kg^{-1}) as well as Pb (0.94 mg kg^{-1}) for T_5. Studies showed that, the accumulation of Pb was high in the root. The highest accumulation of Zn in plant parts was found in stems at T_0 (1.44 mg kg^{-1}). Bioconcentration factors (BCF) and translocation factor (TF) for the three heavy metals (Cu, Pb and Zn) showed that BCF values were lower than 1, while TF above than 1, indicating that *M.*

malabathricum is capable to translocate metals from roots to shoots and this study confirms that this species as a phytoextractor plant.

ABSTRAK

Fitoremediasi ialah penggunaan tumbuh-tumbuhan untuk pemulihan tanah-tanah tercemar dan pendekatan ini tidak diamalkan secara meluas di Malaysia. Kajian ini dilaksanakan di rumah hijau, Tapak Semaian Fakulti Perhutanan, Universiti Putra Malaysia. Tujuan kajian adalah : i) untuk menilai kebolehan *Melastoma malabathricum* mengukur penyerapan logam berat yang bertoksik dari bahan kumbahan dalam medium pertumbuhan selepas tempoh penanaman dan (ii) untuk menilai potensi *M. malabathricum* sebagai tumbuhan *phytoremediator* dalam mengambil logam berat dan translokasi unsur-unsur logam berat di bahagian-bahagian tumbuhan (daun, batang, akar) dengan menggunakan petunjuk Faktor Biokepekatan (BCF) dan Faktor Translokasi (TF). *M. malabathricum* dicambahkan di dalam rumah hijau dalam masa empat (4) minggu dan kemudian diubah ke bahan tanaman (tanah + bahan kumbuhan) untuk tempoh 12 minggu. Anak-anak pokok ditanam pada enam jenis rawatan yang berbeza (tanah + sukatan bahan kumbahan yang berlainan) iaitu; T_0-Pengawal (100% tanah), T_1 (80% tanah dan 20% bahan kumbahan), T_2 (60% tanah dan 40% bahan kumbahan), T_3 (40% tanah dan 60% bahan kumbahan), T_4 (20% tanah dan 80% bahan kumbahan) dan T_5 (100% bahan kumbahan). Setiap jenis rawatan direplikasikan sebanyak empat kali. pH kedua-dua media pertumbuhan telah ditentukan dengan menggunakan kaca elektrod manakala kepekatan logam berat terdiri daripada tembaga (Cu), plumbum (Pb) dan Zink (Zn) dalam media pertumbuhan dianalisis dengan menggunakan spektrometer serapan atom (AAS). Hasil kajian menunjukkan bahawa terdapat kepekatan logam berat di kalangan rawatan sebelum menanam dan selepas penuaian adalah signifikan di mana sebelum menanam kepekatan tertinggi Cu dikesan pada T_5 (3.93 mg kg^{-1}) diikuti oleh T_4 (2.90 mg kg^{-1}), T_3 (2.34 mg kg^{-1}) dan yang paling rendah adalah T_0 (0.91 mg kg^{-1}), manakala selepas penuaian, kepekatan Cu menurun pada semua rawatan. Penurunan tertinggi adalah pada T_5 (1.50 mg kg^{-1}) dan terendah adalah T_1 (0.26 mg kg^{-1}). Bagi Pb kepekatan tertinggi adalah T_5 (9.54 mg kg^{-1}) dan terendah adalah T_0 (0.93 mg kg^{-1}) sebelum tanaman. Selepas penanaman, pengurangan adalah 0.26 mg kg^{-1} bagi T_0 dan 6.30 mg kg^{-1} bagi T_5. Bagi Zn, *M. malabathricum* sangat efektif mengeluarkan logam berat ini terutamanya T_5 iaitu pada tahap pengurangan 14.28 mg kg^{-1}. Pengumpulan tertinggi Cu dalam bahagian tumbuhan adalah akar T_0 (0.13 mg kg^{-1}) serta Pb (0.94 mg kg^{-1}) untuk T_5. Kajian menunjukkan, pengumpulan Pb adalah tinggi dalam akar. Pengumpulan tertinggi Zn di bahagian-bahagian tumbuhan itu ditemui di batang di T_0 (1.44 mg kg^{-1}). Faktor biokepekatan (BCF) dan faktor translokasi (TF) bagi ketiga logam berat (Cu, Pb dan Zn) menunjukan nilai BCF<1 dan TF>1, ini memberi petunjuk bahawa *M. malabathricum* berkupayaan untuk

melakukan proses translokasi logam dari akar ke pucuk dan kajian ini mengesahkan bahawa spesies ini adalah tumbuhan *phytoextractor*.

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APPROVAL SHEET

I certify that this research project report entitled "EVALUATION OF HEAVY METAL UPTAKE BY SENDUDUK (*Melastoma malabathricum*) AS A PHYTHOREMEDIATOR OF SOIL CONTAMINATED WITH SEWAGE SLUDGE" by Shairani b. Mamat has been examined and approved as a partial fulfilment of the requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry, Universiti Putra Malaysia,

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CHAPTER 1

INTRODUCTION

1.1 General Background

Since of the beginning of the industrial revolution, heavy metals contamination has been increasing drastically and gives rise to a serious concerns about the environment. Heavy metal contamination is considered a serious threat to soil and water resources and subsequently, to human health (Yoon et al., 2006). Currently, the expanding human populations combined with the mushrooming of new towns are the main reason of the increasing amount of total domestic and industrial waste. Developed countries with access to good modern technologies have the possibilities to treat their wastes, resulting in the production of sewage sludge. According to Sigh *et al.* (2003), during the last few decades, the amount of heavy metals released into the environment has reached 22,000 tonnes for the cadmium (Cd), 939,000 tonnes of cooper (Cu), 1,350,000 tonnes of zinc (Zn) and 738,000 tonnes of lead (Pb).

Sewage sludge containing various pollutants resulting mainly from anthropogenic and geological activities, poses a serious risk to living. Anthropogenic activities resulting in heavy metal pollution are sewage sludge released from industrial, smelting, mining, military activities, fuels productions and chemicals from the agricultural activities, (Jadia & Fulekar, 2009). Currently, sewage is being remove for example by burning or releasing into the ocean, however, these practices imply negative impacts on the

environment. Therefore, a new environmental friendly technique is needed to efficiently eliminate dangerous waste.

The presence of large amount of heavy metals can negatively affect living organisms in many ways. Previous studies showed that the heavy metals have a care in organic effect. However, at the presence of small amounts of heavy metals can actually be beneficial for humans and plants. Therefore, it is essential to ensure that these heavy metals are being controlled and that they do not negatively affect human food cycle.

Highly toxic elements in waste products may also contain high amounts of organic material and essential nutrients that can enhance plant growth and therefore, they might be potentially used for soil reclamation or soil quality improvement activities.

Several techniques such as physio-chemical and detoxification have been established to remove the heavy metal content in sewage sludge (Ghoosh & Singh, 2005). However, these techniques are not suitable to remove toxic elements from sewage sludge on a large scale because they are costly, time-consuming and potentially accelerate the movement of pollutants (Danh et al., 2009). These techniques are also relatively labour-intense and have potential to destroy soil biotic elements and soil structure. Apart of two techniques, bioremediation has been introduced to remove heavy metals from the soil and to reduce soil toxicity by using micro-organism. However,

this technique was less successful into removal of some type of heavy metals and organic pollutants and also contributed to soil decline in soil quality.

An environmentally friendly and effective approach to eliminate the heavy metals from sewage sludge needs to established and the use of plants can be one way to achieve this (Jadia & Fulekar, 2009). For that purpose, an easy, unique and green-tech based was established to treat polluted areas. The word Phytoremediation is the combination of the Greek words (phyton) meaning plant and *remediate* meaning to preserves. Phytoremediation is defined as the use of various plants in treating soil or water resources that were polluted by waste products, and can be further classified into five types, Phytoextraction, Phytostabilization, Phytodegradation, Phytovolatilization and Rhizofitration.

Studies on phytoremediation are more focused on crops; however recently, studies were conducted on plant species with a high potential to be used as phytoremediators especially in tropical regions where the number of tree species that were identified as phytoextractors are limited. One of the subfields in phytoremediation studies is to select suitable species that can fulfil the phytoremediation requirements for phythoremediation (Macek et al., 2002).

Recently, the potential tropical forest species *Melastoma malabathricum* was identified as a potential phytoremediator. This shrub species is fast growing

which beneficial for phytoremediation, however studies about *M. malabathricum* in regards to phytoremediation scarce.

1.2 Problem Statement

The increasing accumulation of sewage sludge is directly related to an increasing human population. This spiked the need to develop more effective and safe method of disposing sewage sludge. However due to, high amounts of organic matter and nutrients sewage sludge can be used in soil amendments to support plant growth. This method has also been proven to be environmentally friendly, as it can decrease the dependency on organic fertilizers in agriculture.

However, sewage sludge contains heavy metals which can only be removed with difficult and lengthy techniques. The high amounts of heavy metals in sewage sludge have the potential to not only negatively affect crops but also to harm humans consuming these crops. Hence, an environmentally friendly and cost effective method needs to be developed to remove of heavy metal from sewage sludge while maintaining beneficial properties.

As sewage sludge has the potential to simulate plant growth, plants species which are capable of tolerating and accumulating heavy metals can grow successfully in soil amended with sewage sludge. Therefore, one of the most feasible methods of addressing the problem of heavy metals in sewage sludge is phytoremediation. However, not all plants can accumulate or

metabolize heavy metals and currently, only a few tropical tree species have been identified as effective phytoremediators. Therefore, the basic research in the phytoremediation field searches for plant species which are capable of removing heavy metals, resist to highs concentrations of toxic metal. Shrub species are particularly suitable for phytoremediation research due to easy to find, and its fat growing.

For this, I selected *Melastoma malabathricum* and tested its potential as phytoextractor, especially in regards to the following characteristics: rapid growth, high translocation factor, high biomass, good assimilation rate, high tolerance to large amounts of metals, a vast root system and easy to cultivate. Studies to determining the potential for plant species to be used as phytoremediatior are scarce or even lacking.

Up to date, there are no studies looking at the metal accumulation potential of tropical tree species. However, in order to adequately address the increasing volume of domestic and industrial waste in the tropical region, it crucial to identify new metal accumulating tree species.

1.3 Objectives

Several studies have examined the potential of plants species in the phytoremediation of soil concluding that is generally not advisable to use weeds and leafy wild vegetable for phytoremediation as they can easily enter the human food chain and directly or indirectly impact human health.

However, in some tropical countries researched have looked at the phytoremediation potential of local tree species such as Karas (*Aquilaria malaccencis*), Keruing Merah (*Dipetrocarpus verrucosus*), Meranti Paang (*Shorea bracteolate*), and Keruing Kertas (*Dipterocarpus chataceus*). To open up new possibilities, the study examined the phytoremediation of Senduduk (*Melastoma malabathricum*) to remove heavy metal from the sludge. The objectives of this study were:

a) To examine the heavy metal concentrations of sewage sludge in the growth media after harvesting periods.
b) To evaluate heavy metal uptake and translocation in plant parts especially roots, stems and leaves by using an indicator of Translocation factor (TF) and Bio-concentration factor (BCF).

REFERENCES

Abdu, A., Aderis, N., Abdul Hamid, H., Majid, N. A., Muhamad, N., Jusop, S. & Ahmad, K. (2011). Using orthosiphon stamineus B. for Phytoremediation of heavy metals in soils amended with sewage sludge. *American Journal of Applied Sciences*, *8*(4), 323-331.

Ahmadpour, P., Nawi, A. M., Abdu, A., Abdul-Hamid, H., Singh, D. K., Hassan, A., & Jusop, S. (2010). Uptake of heavy metals by Jatropha curcas L. planted in soils containing sewage sludge. *American Journal of Applied Sciences*, *7*(10), 1291-1299.

Artiola, J. F. (1996). Waste disposal. *Pollution Science*, 135-150.

Baker, A. J. (1981). Accumulators and excluders-strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, *3*(1-4), 643-654.

Baker, A. J. M., & Brooks, R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Biorecovery.*, *1*(2), 81-126.

Benavides, M. P., Gallego, S. M., & Tomaro, M. L. (2005). Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology*, *17*(1), 21-34.

Bennett, L. E., Burkhead, J. L., Hale, K. L., Terry, N., Pilon, M., & Pilon-Smits, E. A. (2003). Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. *Journal of Environmental Quality*, *32*(2), 432-440.

Bitton, G. (1999). *Wastewater microbiology, 2nd edition*. New York: Wiley Liss

Brady NC and Weil RR. 2002. *The nature and properties of soils*. New York: MacMillan Publishing Company.

Burken, J. G., Vroblesky, D. A., & Balouet, J. C. (2011). Phytoforensics, dendrochemistry, and phytoscreening: New green tools for delineating contaminants from past and present. *Environmental Science & Technology*, *45*(15), 6218-6226.

Chapman, H. D. (1965). Cation-exchange capacity. Methods of soil analysis. Part 2. *Chemical and Microbiological Properties*, 891-901.

Covington, A. K., Bates, R. G., & Durst, R. A. (1985). Definition of pH scales, standard reference values, measurement of pH and related terminology (Recommendations 1984). *Pure and Applied Chemistry*, *57*(3), 531-542.

Danh, L. T., Truong, P., Mammucari, R., Tran, T., & Foster, N. (2009). Vetiver grass, Vetiveria zizanioides: A choice plant for phytoremediation of heavy metals and organic wastes. *International Journal of Phytoremediation*, *11*(8), 664-691.

Ebbs, S. D., & Kochian, L. V. (1997). Toxicity of zinc and copper to Brassica: species: implications for phytoremediation. *Journal of Environmental Quality*, *26*(3), 776-781.

EPA (2011), Retrieved from: https://www.epa.gov/superfund/students/wastsite/soilspli.htm. Date access: 2 Feb 2016.

Fontes, R. L. F., & Cox, F. R. (1998). Zinc toxicity in soybean grown at high iron concentration in nutrient solution. *Journal of Plant Nutrition*, *21*(8), 1723-1730.

Fresquez, P. R., Francis, R. E., & Dennis, G. L. (1990). Soil and vegetation responses to sewage sludge on a degraded semiarid broom snakeweed/blue grama plant community. *Journal of Range Management*, 325-331.

Ghosh, M., & Singh, S. P. (2005). A review on phytoremediation of heavy metals and utilization of it's by products. *Asian Energy Environment*, *6*(4), 18.

HO, W. M., ANG, L. H., & LEE, D. K. (2008). Assessment of Pb uptake, translocation and immobilization in kenaf (*Hibiscus cannabinus L.*) for phytoremediation of sand tailings. *Journal of Environmental Sciences*, *20*(11), 1341-1347.

Jadia, C. D., & Fulekar, M. H. (2009). Phytoremediation of heavy metals: Recent techniques. *African Journal of Biotechnology*, *8*(6).

Justin, V., Majid, N. M., Islam, M. M., & Abdu, A. (2011). Assessment of heavy metal uptake and translocation in *Acacia mangium* for phytoremediation of cadmium contaminated soil. *Food Agriculture Environment Journal*, *9*, 588-592.

Kabata-Pendias, A., & Pendias, H. (1984). *Trace Elements in Soils and Plants*. Boca Raton, Florida: Lewis Publishers.

Kim, I. S., Kang, K. H., Johnson-Green, P., & Lee, E. J. (2003). Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction. *Environmental Pollution*, *126*(2), 235-243.

Kvesitadze, G., Khatisashvili, G., Sadunishvili, T., & Ramsden, J. J. (2006). *Biochemical mechanisms of detoxification in higher plants: Basis of phytoremediation*. *Springer Science & Business Media*.

Lane, S. D., & Martin, E. S. (1977). A histochemical investigation of lead uptake in *Raphanus sativus*. *New Phytologist*, *79*(2), 281-286.

Lide (1992). *CRC Handbook of Chemistry and Physics (3rd Edition).* Boca Raton, Florida: CRC Press.

Macek, T., Macková, M., Pavlíková, D., Száková, J., Truksa, M., Cundy, A. S., & Scouten, W. H. (2002). Accumulation of cadmium by transgenic tobacco. *Keynote Lectures*, 42.

Marcel, V. D. P. (2006). *Soil and water contamination from molecular to catchment scale.* Taylor & Francis Group, London: Lewis Publisher, pp. 89-135.

Maryam, G., Majid, N. M., Islam, M. M., Ahmed, O. H., & Abdu, A. (2015). Phytoremediation of copper-contaminated sewage sludge by tropical plants. *Journal of Tropical Forest Science*, 535-547.

McGhee, T. J. (1991). Water supply and sewerage. *McGraw-Hill series in water resources and environmental engineering*.

McGrath, S. P., & Zhao, F. J. (2003). Phytoextraction of metals and metalloids from contaminated soils. *Current Opinion in Biotechnology*, *14*(3), 277-282.

Meyer, K. (2001). Revision of the Southeast Asian genus Melastoma. *Blumea*, *46*(2), 351-398.

Mitch, M. L. (2002). Phytoextraction of toxic metals: A review of biological mechanism. *Journal of Environmental Quality*, *31*, 109-120.

Nasir, J., Floresco, S. B., O'Kusky, J. R., Diewert, V. M., Richman, J. M., Zeisler, J., & Hayden, M. R. (1995). Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioural and morphological changes in heterozygotes. *Cell*, *81*(5), 811-823.

Pengnoo, A., Hashidoko, Y., Onthong, J., Gimsanguan, S., Sae-ong, M., Shinano, T., & Osaki, M. (2007). Screening of phosphate-solubilizing microorganisms in rhizosphere and rhizoplane of adverse soil-adapting plants in Southern Thailand. *Tropics*, *16*(1), 1-7.

Polprasert, C., Udom, S., & Choudry, K. H. (1984). Septage disposal in waste recycling ponds. *Water Research*, *18*(5), 519-528.

Pongrácz, E., & Pohjola, V. J. (2004). Re-defining waste, the concept of ownership and the role of waste management. *Resources, Conservation and Recycling*, *40*(2), 141-153.

Purakayastha, T. J., & Chhonkar, P. K. (2010). Phytoremediation of heavy metal contaminated soils. In Soil Heavy Metals. *Springer Berlin Heidelberg*, 389-429.

Rajenderan, M. T. (2010). Ethno medicinal uses and antimicrobial properties of *Melastoma malabathricum*. *SEGi Review*, *3*(2), 34-44.

Rajoo, K. S., Abdu, A., Singh, D. K., Abdul-hamid, H., Jusop, S., & Zhen, W. W. (2013). Heavy metal uptake and translocation by *Dipterocarpus verrucosus* from sewage sludge contaminated soil. *American Journal of Environmental Science*, *9*(3), 259-268.

Raskin, I., & Ensley, B. D. (2000). *Phytoremediation of toxic metals: Using plant to clean up the environment* (1st edition). New Jersey, US: Wiley-Interscience.

Reeves, R. D., & Baker, A. J. M. (2000). *Metal-accumulating plants. Phytoremediation of toxic metals: Using plants to clean up the environment*. New Jersey, US: Wiley Interscience, 193–229.

Reichman, S. M. (2002). *The Responses of Plants to Metal Toxicity: A Review Forusing on Copper, Manganese & Zinc*. Melbourne, Australia: Australian Minerals & Energy Environment Foundation, 22-26.

Romeiro, S., Lagôa, A. M., Furlani, P. R., Abreu, C. A. D., Abreu, M. F. D., & Erismann, N. M. (2006). Lead uptake and tolerance of *Ricinus communis L. Brazilian Journal of Plant Physiology*, *18*(4), 483-489.

Rosazlin, A., Fauziah, C. I., Rosenani, A. B., & Zauyah, S. (2007). Domestic sewage sludge application to an acid tropical soil: part III. Fractionation study of heavy metals in sewage sludge and soils applied with sewage sludge. *Malaysian Journal of Soil Science*, *11*, 81-95.

Rostagno, C. M., & Sosebee, R. B. (2001). Surface application of biosolids in the Chihuahuan Desert: Effects on soil physical properties. *Arid Land Research and Management*, *15*(3), 233-244.

Rufus L.C. (2000). Phytoremediation: Using Plants to Clean Up Soils: *Agricultural Research Magazine 48*(6), 4-9

Salt, D. E., Blaylock, M., Kumar, N. P., Dushenkov, V., Ensley, B. D., Chet, I., & Raskin, I. (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Nature Biotechnology*, *13*(5), 468-474.

Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., & Jain, R. K. (2003). Phytoremediation: An overview of metallic ion decontamination from soil. *Applied Microbiology and Biotechnology*, *61*(5-6), 405-412.

Surriya, O., Saleem, S. S., Waqar, K., & Kazi, A. G. (2015). Phytoremediation of soils: Prospects and challenges. *Soil Rem Plants-Prospects Chall*, *1*, 1-36.

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, *2011*.

Tchobanoglous, G. (1987). *Aquatic plant systems for wastewater treatment: engineering considerations.* Orlando, Florida: Magnolia Publishing Inc, *27-48*

US-EPA (2001). Pb, identification of dangerous levels, final rule. Code of Federal Register, (66): 1206-1240.

Waqar, A., Ullah, N., and Munir, H.Z. (2015). Soil contamination with metals: Sources, types and implications. Soil Remediation and Plants. *Elsevier,* 37-61.

Water UK (2006). *Wastewater treatment and recycling*. Retrieved from: http://www.water.org.uk/publications/reports/wastewater-treatment-and-recycling-pamphlet. Date access: 6 Feb 2016.

Werther, J., & Ogada, T. (1999). Sewage sludge combustion. *Progress in Energy and Combustion Science*, *25*(1), 55-116.

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecology*, *2011*.

Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *The Science of the Total Environment*, *368*(2-3), 456–64.

Zarcinas, B. A., Ishak, C. F., McLaughlin, M. J., & Cozens, G. (2004). Heavy metals in soils and crops in Southeast Asia. *Environmental Geochemistry and Health*, *26*(4), 343-357.

Zhou, L. X., & Wong, J. W. C. (2001). Effect of dissolved organic matter from sludge and sludge compost on soil copper sorption. *Journal of Environmental Quality*, *30*(3), 878-883.

Zhou, Q. X., & Song, Y. F. (2004). Principles and methods of contaminated soil remediation. *Chin Environ Sci Press, Beijing*: 568.