TOXICITY STUDY OF ARSENIC AND NICKEL IN POTENTIAL MYCOREMEDIATING FUNGI

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ABSTRACT

Pollution is getting more and more severe due to the anthropogenic discharge of harmful chemical such as heavy metal contamination such as arsenic, nickel. When the concentration of heavy metals are too high, they will bring harmful effects to those living organisms that are exposed for a long duration of time like plants, animals and human health. Bioremediation such as mycoremediation is a preferable method to remove these pollutants because it is cheap, environmental friendly and it is most likely to completely degrade pollutant. The objective of this research study is to identify the potential fungi in remediating nickel (Ni) and arsenic (As) pollution based on their tolerance level to the heavy metal. Therefore, different concentration of As and Ni is used to test the tolerance level of the fungal and each fungal have three replicate. The potential fungi that were tested to remediate As and Ni are *Trichoderma longibrachiatum*, *Hypocrea koningii*, *Penicllium simplicissimum*, *Penicillium chrysogenum* and *Aspergillus ustus*. The total growth of fungi was determined based on their dry weight (g/weeks). One way analysis of variance (ANOVA) with PostHoc tests (LSD and Dunnett T3) in SPSS software were used to analyse the growth rate of fungi. The highest tolerance to As and Ni was *Aspergillus ustus* (up to 20ppm with biomass of 0.09 g/weeks).The lowest tolerance to As and Ni was *Trichoderma longibrachiatum* (up to 20ppm with biomass of 0.03 g/weeks).

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LIST OF ABBREVIATIONS

ppm	Particles per million
g	Gram
mL	milliliter
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
As	Arsenic
LAF	Laminar Air Flow
Ni	Nickel
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of variance
g/week	Gram per week
T. longibrachiatum	*Trichoderma longibrachiatum*
H. koningii	*Hypocrea koningii*
P. simplicissimum	*Penicillium simplicissimum*
P. chrysogenum	*Penicillium chrysogenum*
A. ustus	*Aspergillus ustus*
°C	Degree Celsius

CHAPTER 1

INTRODUCTION

Recently, the environmental contamination due to heavy metals has caused the ecological and global public health concern to increase rapidly (Raymond, 2011). More and more humans are found to be exposed to these heavy metals due to the severe increase in activities from several fields such as agricultural, technology and industrial. These activities are cause environmental contamination such as atmospheric deposition, metal corrosion, leaching of heavy metals, and metal evaporation from water resources to soil or ground water and soil erosion of metal ions and they can potentially endanger the human health (Aleksandra, 2014).

One of the heavy metals that bring harmful effects to human health is arsenic (As). As is an extensively dispersed metalloid which can normally be found in groundwater used for drinking purposes in certain countries throughout the world like Chile, China and Bangladesh (Lars, 2003). Other than groundwater, As can also be found in the air whereby the concentrations in the provincial areas range from <1 to 4 ng/m^3, while the air concentration in the cities can be as immense as 200 ng/m^3 (Berglund, 2001). According to Lars (2003), a much higher concentration (>1000 ng/m^3) of As is measured nearby the industrial area which can lead to serious environmental pollution that can harm human health. In Malaysia, there are currently no extensive soil reference values available to inaugurate levels of potentially lethal As for distinct land usage such as residential, agricultural, recreational land and industrial (Nordberg G,2011).

Another heavy metal that can affect human health is nickel (Ni). Ni is an element present in the environment at very submissive levels which plays an important role in small doses. However, it can be very dangerous when it exceeds the maximum tolerable amount. According to Kabata- Pendias and Pendias (1992), the Ni content in soils diverse broadly and have been estimated to range between 3 and 1000 ppm. On the other hand, for the world soils, the range is from 0.2 to 450 ppm (Kabata- Pendias and Pendias, 1992). In Malaysia, the levels of Ni in clams from

Sungai Sepang (Selangor), Pantai Remis (Selangor), Kampung Pasir (Johor), Parit Jawa (Johor), and Pantai Remis (Perak) are higher than 5.00 μg/g (Rezeaur, 2015). The cause may be that human activities such as metal mining, smelting, refining, fossil fuel combustion, and solid waste disposal are the significant sources of this metal discharge to the environment and large amount may be transferred to marine environment through municipal sewage effluent containing industrial waste (Sinin Hamdan, 2015). As an example, serious contamination by heavy metal such as Nickel and Arsenic has also been found in Klang Valley due to the irresponsible industries disposing heavy metal inappropriately, open burning and land transportation (Rafia, 2003).

Currently, the best demonstrated available technologies (BDATs) for remediation of heavy metals in contaminated sites are bioremediation, immobilization and soil washing (Raymond, 2011). Optimal technology that can be used to remediate heavy metals is bioremediation (Anastasi, Tigini, & Varese, 2013). Bioremediation is the process using living organism to remove toxic contaminant from groundwater. Therefore, in this study, mycoremediation which is the subdivision of bioremediation was used to remediate As and Ni through the usage of fungi by determining the tolerance level of potential fungi namely against different concentration of Ni and As. The objective of this study is to determine the potential of mycoremediation in remediating nickel and arsenic based on their tolerance level.

CHAPTER 2

LITERATURE REVIEW

2.1 HEAVY METAL

2.1.1 Arsenic

Arsenic is classified as a semi-metal and also a natural element. It is grey in colour when its form is pure and it is usually discovered in combination with elements like silver, copper, gold and lead. It comes naturally from the Earth's crust discovered generally in soil, rock, air and water (Chai, 2013). Human activities such as industrial operations and metal mining can increase the presence of arsenic. It can be spread into the air and eventually deposited on surface soil and water (Rudra, 2007). Arsenic is commonly discovered in inorganic form from rocks, soil, industrial waste, surface water and ground water. However, organic arsenic can be discovered in in animals, fish, plants and pesticides (Franz, 1991).

2.1.2 Background of Arsenic

The abundance of arsenic globally is generally 1.5-2ppm. It is present in minerals and soil and it may enter water, land and air through water run-off and wind-blown dust (Stollenwerk, 2007). Arsenic is present in the atmosphere come from different sources: microorganisms discharged 20.000 tonnes of volatile methylarsines per year and volcanos discharged around 3000 tonnes yearly, whereas human activity has to be responsible for much more: 80.000 tonnes of arsenic yearly are released through the burning of fossil fuels (Grimm, 2005).

Marine organisms usually contain arsenic residues ranging from lower than 1 to higher than 100 mg/kg (Koodkaew, 2012). Arsenic can multiply (bioaccumulate) in aquatic organisms bodies, especially those that live in the sea. The concentration of arsenic in land-living and freshwater plants and animals are normally less than 1 mg/kg. The land-living plants can accumulate arsenic by deposition of arsenic in the air on the leaves or through uptake by roots from the soil (Fourest, 1992). Living organisms in areas that has volcanic activity or near man-made sources of arsenic tends to have higher arsenic levels. Around 3000 mg/kg has been discovered in several species at the arsenical mine sites (Howell, 2003)

The arsenic concentration discovered in severely contaminated drinking water in Bangladesh is ranged from 170 to 1500 micrograms per litre (A microgram is a millionth of a gram). In contrary, to be fatally poisoned a person; he/she would has to ingest more than 70,000 micrograms of arsenic at once in a single dose (Andrew, 2010). Nonetheless, exposure to arsenic concentrations like those found in Bangladesh over a long period will correlate to a vast range of illnesses. Most of the world's safe drinking water standards for arsenic are currently based on risk estimation using data on those people who are exposed to immensely high levels of arsenic through their workplace or through drinking water in areas like Taiwan, Bangladesh and parts of South America (Cempel, 2006).

There are also several studies that has examined the effects of minor doses on people over a long duration of time. The researchers from Dartmouth are coordinating epidemiological studies to find out the health effects of drinking water with presence of arsenic at elevated levels discovered in several parts of the United States (Keeve, 2016). The levels which are typically between 50 to 200 micrograms per litre are very much lower compared to those of Bangladesh (Morin, 2006). However, they are still considered high enough to be of concern.

2.1.3 Effect of Arsenic

Exposure to arsenic in drinking water for long term can lead to cancer in the lungs, skin, kidney and bladder (Barazani, 2004). Besides, it can also cause other changes in the skin such as pigmentation and thickening. The tendency of the effects is correlated to the level of arsenic exposure and in areas where drinking water is severely contaminated; these effects can be noticed in lots of individuals in the population. People who ingest arsenic in drinking water at 50 µg/litre or lower than 50 µg/litre concentrations has been reported to have increased risks of bladder and lung cancer and also skin changes (Das, 2007). Other than that, inhalation of arsenic in the workplace can also lead to lung cancer. The tendency of cancer was associated to the duration and level of exposure (Sabino, 2016). Exposure levels of more than 750 µg/m^3 per year had been detected to increase the risk of lung cancer (Xu, 2017). However, over exposure of arsenic in plant will lead to lethality, inhibition of growth, reproduction and photosynthesis. Hence, the dynamic of food chain will be affected.

2.1.4 Nickel

Nickel is a lustrous and naturally occurring silvery-white element. It appears extensively in earth's crust and it is also the fifth most common element on earth. Nickel is hard, silvery-white, ductile metal and malleable (Dhankhar, 2011). It is present in water, air and soil in different form. Natural sources of atmospheric nickel levels include wind-blown dust, derived from volcanic emissions, weathering of soils and rocks, vegetation and forest fires (Deng, 2013). Nickel compound and nickel itself has many commercial and industrial used where the progress of industrialization has cause the emission of pollutants into the ecosystems to increase (Ting, 2009). Despite Ni is ubiquitous and important for the role of many organisms, the concentrations in certain areas from both naturally varying levels and anthropogenic released can be toxic to living organisms.

2.1.5 Background of Nickel

The content of nickel in soils diverse widely and has been approximated to range between 3 to 1000ppm; whereas the world soils brand range is from 0.2 to 450ppm and the grand mean is computed to be 22ppm (KabataPendias and Pendias, 1992; Cempel and Nikel, 2005; Bencko, 1983; Scott-Fordsmand, 1997). Duke (1980a) also disclosed an average concentration for the natural nickel content in the earth's crust is 86 ppm. Chen et al (1999) has reported the nickel contamination level in rural soils for several countries which are China (20 ppm), Australia (60 ppm), France (50 ppm), United Kingdom (60 ppm), Japan (100 ppm), South Africa (15 ppm), Germany (200 ppm), Netherland (210 ppm), United State of America (420 ppm) and Canada (150 ppm). Other than that, Boerngen and Shacklette (1984) reported the concentration of Nickel for the soil survey in the United States range from less than 5 to 700ppm.

There are various anthropogenic activities such as metal mining, fossil fuel burning, organic manures, smelting, municipal and industrial wastes, and vehicle emissions, application of fertilizer and disposal of household which causes the release of Nickel into the environment (Harman, 2004). Besides, the main cause of nickel present in drinking water is from metals that are in contact with drinking water like pipes. Nickel may also be found in some ground water due to the consequence of dissolution from nickel ore bearing rocks (Urik, 2007).

2.1.6 Effect of Nickel

The primary cause for nickel-induced toxicity is the inhalation exposure by human during their workplace setting which can lead to toxic effects to the immune system and the respiratory tract (Cempel, 2005). The general population's exposure to nickel is primarily from oral intake, mainly through food and water as they are contaminated. However, the individuals who are not occupationally exposed can also be affected by everyday used equipment such as nickel-plated articles and stainless steel as they are a common sensitizing agent that has high extensiveness of allergic contact dermatitis (WHO, 2005). When the skin is in contact with nickel- contaminated water or soil, it may result in nickel exposure (Yamada, 2001)