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Preliminary Test of Mercury Exposure on *Paspalum vaginatum* in Phytoremediation Process

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Abstract : In this study, the range of mercury (Hg) concentration in *Paspalum vaginatum* was determined using a preliminary toxicological test. The test was conducted in a greenhouse for 28 d to observe the level of Hg contamination that affected the test plants. One of the observations made was whitering of plants. This indicated that the plants have been affected by the dosage used. Pots containing 3 kg of sand contaminated with different Hg concentrations [0 (control), 0.5, 1, 2, 4, 6, and 8 mg/L] were used. Observation was made thrice a week. After 28 d of observation, the plant species grew and survived in pots with Hg concentrations of 0.5, 1, and 2 mg/L. The preliminary test showed that *P. vaginatum* can treat sand with contaminated Hg. The results of this study may serve as a basis for research that aims to study uptake and accumulation of Hg using potential phytoremediation plants.
Keywords: mercury, range-finding test, spiked soil, *Paspalum vaginatum.*

Introduction

Mercury (Hg) is considered as a global pollutant because of its tremendous potential for biological transformation (into harmful forms), bioaccumulation, and biomagnification through ecological food chains [1]. Hg emissions into the atmosphere come from both natural and anthropogenic sources. Natural sources usually include volcanic eruptions, geothermal activities, forest fires, and soil and water surface evaporation, whereas anthropogenic sources include fossil fuel combustion, waste incineration, chlor-alkali plants, and metallurgical processes[2-4].

Hg is present in numerous chemical forms. It naturally occurs in the environment as mercuric sulphide. Elemental Hg itself is toxic and cannot be broken down into less hazardous compounds, whereas elemental or inorganic forms, especially methylated ones, can be transformed into organic forms by biological systems. These methylated mercurial compounds are not only toxic but are also highly bio-accumulative[5].

Hg is present in a wide array of chemical and biological transformation processes, such as Hg^0 oxidation and Hg^{2+} reduction or methylation, depending on soil pH, temperature, and humic content[6, 7]. The formation of organic Hg^{2+} complexes is known to be the dominating process, which is largely attributed to the affinity of Hg^{2+} and its inorganic compounds to sulfur (S)-containing functional groups[6]. In soils low in organic matter, most Hg can be found as reactive, ionic Hg species, such as $HgCl_2$ or $Hg(OH)_2$, which can be transformed easily into more toxic forms, such as methyl Hg or Hg^{08}.

The biscuit grass *Paspalum vaginatum*, which usually grows in coastlands, estuarine habitats, and wetlands, is the plant used in this research. This species is a local grass plant that belongs to the Poaceae family[9]. Grasses are reportedly the best plants to be used in contaminated soil remediation because of their fibrous root systems with extensive surface area for microbial colonization[10]. The fibrous root system forms a continuous, dense rhizosphere, providing ideal conditions for phytoremediation.

Range-finding phytotoxicity test is a preliminary test performed to establish the definitive test for the main toxicology experiment[11]. It determines the contaminant concentration at which the plant species can survive. The parameter used for the acute toxicity test measurement includes the number of individual mortality or death inhibitory effect on plant growth. It is indicated as LC50 (lethal concentration) or EC50 (effect concentration). LC50 is the concentration of a given agent that is lethal to 50% of the tested organisms, whereas EC50 is the concentration of a given agent that induces a response to 50% of the organisms[12, 13]. Both are estimated by graphical or computational means.

In this study, a preliminary test for the phytoremediation of Hg-contaminated soil was conducted using *P. vaginatum*. This study aims to determine the maximum Hg concentration at which *P. vaginatum* can survive. The results of this study may serve as a basis for further research focusing on identifying plants that can be used for the phytoremediation of Hg-contaminated soil.

Materials and Methods

Preparation of Hg-spiked sand

The experiment was conducted in a greenhouse in UKM, Malaysia using Hg-spiked sand. Sand was sieved (5 mm in diameter) to remove coarse fragments and obtain uniform size [14, 15]. The range-finding test was conducted on a single exposure system with several Hg concentrations [0 (control), 0.5, 1, 2, 4, 6, and 8 mg/L] prepared by diluting analytical-grade $HgCl_2$ in deionized water. Each pot containing 3 kg sand spiked with Hg was planted with three plants. The number of withered plants was observed within 4 weeks. The amount of solution used was determined by referring to the bulk density of the soil. Therefore, the ratio of soil to the water was 100 g: 26 ml. For 3 kg of soil, the final volume of the solution should be 780 ml. This ratio also used to determine the amount of water for watering the plant. The volume of contaminant for each concentration was determined by using dilution method with formula:

$C_1V_1=C_2V_2$

where V_1 is the volume of stock solution, C_1 is the concentration of stock solution, V_2 is the volume of water in the reactor, and C_2 is the concentration of Hg required.

The range-finding phytotoxicity test was conducted for 28 d. Watering was conducted every 3 days.

Plant growth observation

The preliminary test was conducted to physically observe the level of Hg contamination at which the plants can grow and survive. The observation was conducted thrice a week for 28 d. The number of withered plants was observed every 3 d. The percentage of withered plants in each concentration was determined relative to the total number of plants in the pot. The number of withered plants was recorded, and their percentage was determined using Eq. 1:

$$Withered\ plant\% = \frac{No.of\ withered\ plant}{No.of\ total\ plant} \times 100$$

The concentration-response curve describes the response of cumulative effect to a range of Hg concentration [12]. The cumulative effect (wilting, drying, and death) to the plant as a response versus Hg concentration is drawn, and the Hg concentration that results in 50% of the measured effect can be determined from the graph.

Statistical analysis

Results for pH and temperature are reported as mean ± standard deviation. Regression analysis for the determination of the LC_{50} was performed using the Microsoft Excel software. The LC_{50} was calculated using the equation determined by the software for the regression curve.

Results

The preliminary test observations were performed by determining the effects of toxic Hg on the physical plant (wilted or dried). Fig.1 (a-v) summarizes the observation of *P. vaginatum* during the range-

finding phytotoxicity test. The withering effects observed on different leaf colors changed compared with the normal green color of the leaves. The leaves wilted and changed color to brown.

Figure1(a-v): Growth of *P. vaginatum* in different mercury concentrations

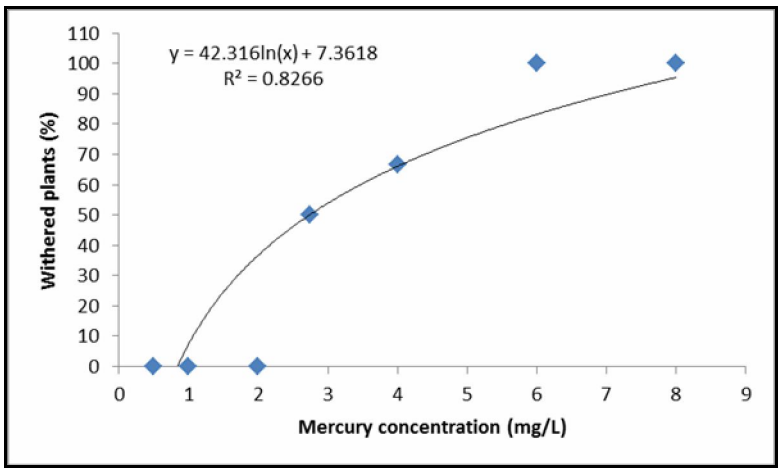

Figure 2: toxicity *P. vaginatum* concentration-response curve at day 28

The observation was done daily for 7 d and then 3 times a week for 4 weeks. The dose-response curve plotted as a cumulative number of withered plants by each mercury concentrations using is represented (Fig. 2).The concentration effect of the mercury on the withered percentage of *Paspalum vaginatum* correlated well as a linear regression (R^2=0.83). Test results showed that the range of 50% withered plants appears approximately at 2.74mg/L mercury concentration. After 28 day of observation, the minimum percentage of withered plant at 4 mg/L concentration was 66.6%, and the maximum percentage at 6 and 8 mg/L concentrations was 100% (Fig. 2). Therefore, Hg concentrations with less than 4 mg/L will be considered for future phytotoxicity studies to ensure the plant is able to survive. The plants in Hg concentrations ranging from 0.5 mg/L to 2 mg/L could still survive and adapt to the environment. Severe visual toxic symptoms, such as weathering and chlorosis, were observed, especially at 8 mg/L Hg concentration (Fig. 3a-c).

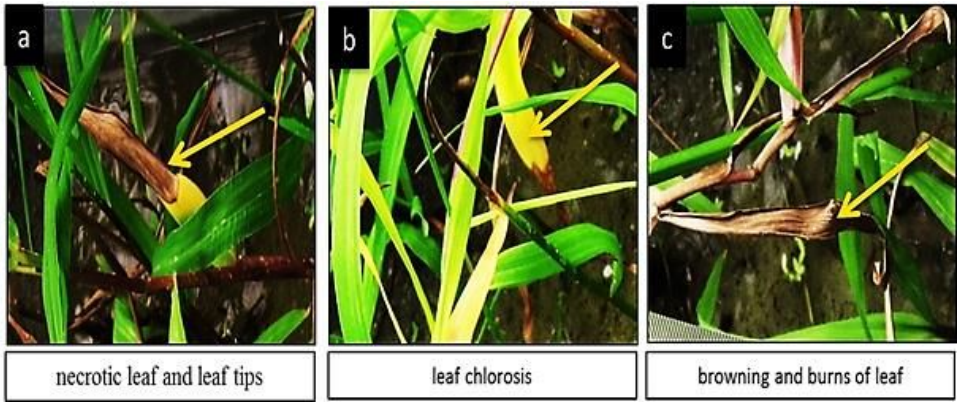

Figure 3(a-c): Effect of mercury on leaves of *P. vaginatum*. Arrows show mercury induced toxic effects.

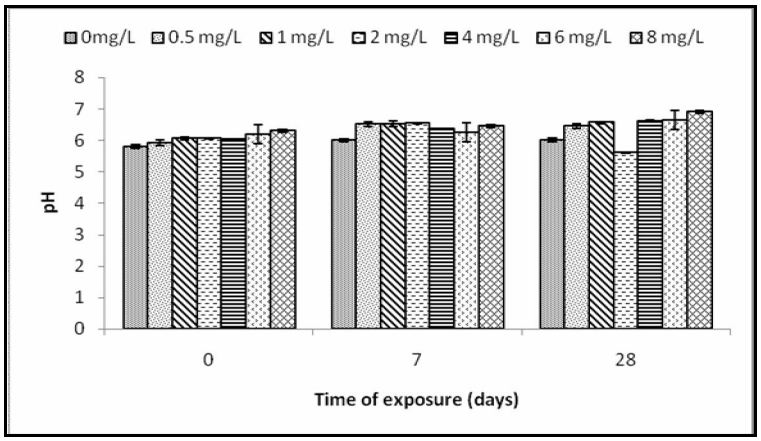

Figure 4: pH spiked sand during Hg preliminary test of *P .vaginatum*

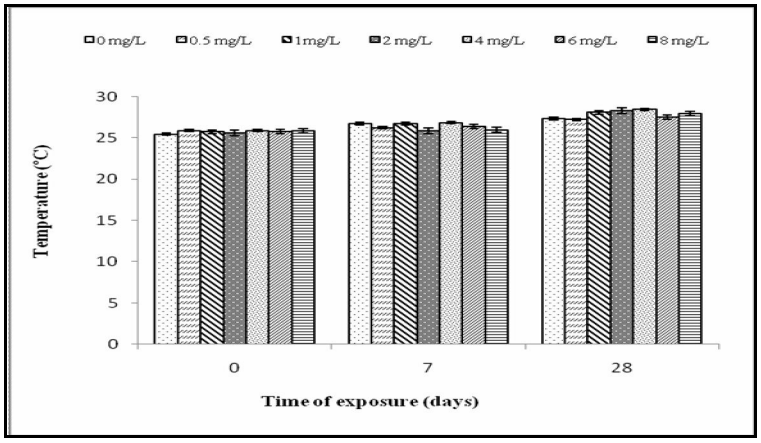

Figure 5: Temperature spiked sand during Hg preliminary test of *P .vaginatum*

Physical parameters (T and pH) were recorded throughout the range finding phytotoxicity test, for *P .vaginatum*. In general, the results show that the pH mean values ranged between 5.8±0.05 to 6.9±0.04 (Fig.4) throughout the the range finding phytotoxicity test; this value were within still the range of pH (5-7.5) as mentioned in OECD (1984).Temperature is 25.5±0.16-28.45±0.14 °C (Fig. 5), this is a normal at tropical climate.

Discussion

The effects of Hg on plants were well documented[16,17]. Hg harms plants through impairment of the synthesis and metabolism of chlorophyll[18,19], chromosome damage (Panda et al. 1992), and inhibition of root and shoot growth [20]. These effects may result in visible symptoms of stress, including leaf chlorosis, necrosis, and stunted growth [17, 21].Hg inhibits water uptake through aquaporins in plasma membranes in wheat[22]. It can also rapidly and significantly decrease pressure-induced root water flux in tomato plants and reduce 57% of root system hydraulic conductivity[23].Suszcynsky and Shann [24]showed that inhibition of root and shoot growth occurs at 1.0>µg/mL Hg, with very limited tissue damage at higher levels of treatment. Moreover, Hginduced root damage may have serious consequences for nutrient and water supply to aboveground plant parts [20].The critical levels of Hg toxicity in plant tissues range from 0.5 ppm[25] to 3ppm[26, 27], but depend on plant species and age.

Mhatre and Chaphekar[28] observed damage to plants even at 1 µg/L Hg concentration in the nutrient solution. They reported that Hg impact should be considered in events of failure of various metabolic processes such as photosynthesis, chlorophyll manufacturing, and exchange of gases and respiration. Higher concentrations (>1 mg/L to 2 mg/L) of Hg decrease the growth of tobacco[24], tomato[19], and alfalfa[29].

Conclusions

Through a range finding test, of 28 days Hg exposure, results were showed that as the mercury concentration increased the increase in number of plants withering was observed. This study demonstrated that *P. vaginatum* has the ability to survive up to the Hg concentration of 2 mg /L in the range finding phytotoxicity test. Hence, the future phytotoxicity test will be conducted on 0.5 – 2 mg/L contaminant concentration of mercury.

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