The effectivity of Biduri combined with indigenous bacteria in mercury absorption

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Abstract. Heavy metals pollution, especially Mercury (Hg), is one of the most serious environmental problems. The presence of excessive Hg will cause soil degradation and threaten the life of the ecosystem, for that remediation is necessary. Biduri is known to be able to absorb heavy metals, but there is no research on the ability of Biduri in absorb Hg. The use of indigenous bacteria is expected to increase the absorption of Mercury by Biduri. The purpose of this study was to determine the potential of Biduri combined with indigenous bacteria and Agrobacterium sp I37 in absorbing of Hg in the soil. The experimental was designed as factorial with completely randomized design, consisting of 2 factors namely Bioremediation agent (A0: without bioremediation agent, A1: indigenous bacteria, A3: Agrobacterium sp I37) and Hg dosage (D0: without Hg, D1: Hg 0.3 µg.g-1, D2: Hg 0.51 µg.g-1). The results showed that the combination of Biduri with indigenous bacteria + 0.3 µg.g-1 Hg shows highest absorption of Hg at 57.19 µg (99.24% higher than control) and reduce soil Hg levels by 0.09 µg.g-1. Biduri is a hyperaccumulator plant because it is able to absorb more than 10 µg.g-1 of mercury.

1. Introduction

Mercury (Hg) is a heavy metal which often used in for gold mining in Jendi Wonogiri. The method known as amalgamation, where Hg is used to extract gold from the ore [1]. When Hg mixed with the ore, it forms an amalgam with gold or silver. To get gold and silver, the amalgam must be burned to evaporate the Hg, then Hg would pollute the environment. The artisanal gold miners use mercury to capture and separate the gold grains from the rock grains. This Hg precipitate was filtered using a cloth to get the gold. The filtered precipitate is then kneaded by hand. Mining wastewater containing Hg is allowed to flow into rivers which are used as irrigation water, then it has potential to enter the food chain. The environment polluted by Hg can endanger the lives of living things because Hg enters the body through the food chain cycle [2]. Mercury is toxic and accumulates in small quantities. Mercury is absorbed in the body in the long term and will be harmful to human health [2]. The dangers of diseases caused by mercury compounds include damage to hair, teeth, memory loss and disruption of the nervous system [3]. Mercury is a toxic element that is as global concern because it poses a danger to humans, animals and ecosystems [4].

Thirty soil samples collected in area of Jendi Village contained Mercury with an average of 30.87 µg.g-1. Based on Government Regulation No. 101 of 2014 concerning Management of Hazardous and Toxic Waste, the threshold for mercury levels in the soil is 0.3 µg.g-1 [1]. Mercury levels in river water around Jendi Village vary from 0.0024 mg/l to 0.0173 mg/l. Government Regulation No. 82 of
Concerning Water Quality Management and Water Pollution Control, the mercury threshold in water is 0.001 mg/l. Thus, mercury levels in the soil and river water in Jendi Village have exceeded the predetermined threshold. Therefore, it is necessary to do bioremediation as an effort to reduce pollutants in the environment by utilizing living things. One form of bioremediation is phytoremediation, which uses plants to clean the environment of pollutants [5]. Biduri (*Calotropis gigantea* (L.) W.T. Aiton) is known to be able to absorb Cadmium (Cd) at the roots of 1.26 µg.g⁻¹ and at the shoots of the plant 1.01 µg.g⁻¹, higher than elephant grass. The level of Cd reduction by Biduri plants was 64.76% higher than elephant grass [6]. Biduri has properties such as resistance to drought and high salinity, producing fiber, and potential for bioremediation by minimizing by-products [7].

The following is Biduri classification,

| Division       | Magnoliophyta                   |
|----------------|---------------------------------|
| Class          | Magnoliopsida                   |
| Ordo           | Gentianales                     |
| Family         | Asclepiadaceae                  |
| Genus          | Calotropis R. Br.               |
| Spesies        | *Calotropis gigantea* (L.) W.T. Aiton [8] |

Biduri in this study was combined with bacteria, namely *Agrobacterium* sp I37 and indigenous bacteria. *Agrobacterium* sp. I37 was able to reduce Pb in the soil by 42.91% from 8.04 µg.g⁻¹ to 4.59 µg.g⁻¹, was able to increase Pb accumulation in roots by 69.63% and reduce Pb levels in rice grains by about 55.67% more higher than the control treatment [9]. According to Franchi et al. [10] indigenous bacteria can increase phytoextraction by 85% for As and 45% for Hg. The use of indigenous bacteria was isolated from the people's gold mining in Jendi village, Selogiri District, Wonogiri Regency. To determine the potential of Biduri in absorbing Hg, it was done by adding different doses of mercury to the experimental pot. The purpose of this study was to determine the potential of Biduri in absorbing Hg either alone or in combination with indigenous bacteria and *Agrobacterium* sp I 37 in soil treated with Hg (contaminated with Hg).

2. Materials and methods

2.1. Area study

This research was conducted from May to November 2020 at the Greenhouse of the Faculty of Agriculture, Sebelas Maret University (UNS). Analysis of soil samples and plant samples was carried...
out at the Laboratory of Soil Biology and Biotechnology, Laboratory of Chemistry and Soil Fertility, UNS. Heavy metal analysis was carried out at the UPT UNS Laboratory.

2.2. Materials and equipment
The materials used include soil as a planting medium obtained from Ngargoyoso Village, Tawangmangu, Karanganyar, indigenous bacteria isolated from the community gold mine in Jendi Wonogiri [11], Agrobacterium sp I3 isolated by Rosariastuti et al. [12], Biduri plant seeds from Balai Besar Tekstil, and Nutrient Agar (NA) medium with the composition of beef extract 10 g/l, peptone 10 g/l, NaCl 5 g/l, 1,000 ml aquades, and 15 g agar/l. Nutrient Broth (NB) with a composition of beef extract 3 g/l and peptone 10 g/l [10]. The equipment needed is a sample ring, Erlenmeyer, pH meter, electric oven, refrigerator generator, Analytical Balance, hot plate and stirrer, Autoclave, vortex, shaker, fume hood, and Mercury analyzer.

2.3. Experimental Design
The experimental design was factorial with a completely randomized design as the basic design, consisting of 2 factors: bioremediation agent (A0: without bioremediation agent, A1: with indigenous bacteria, A2: with Agrobacterium sp I37), and Mercury dose (D0: without Mercury, D1: with mercury 0.3 μg.g⁻¹, B2: with mercury 5.15 μg.g⁻¹). In total there are 9 treatment combinations, each repeated 3 times, then resulted 27 experimental units.

2.4. Procedure
2.4.1. Bacterial inoculum. Bacterial inoculum preparation using 1 slanted media containing isolates of Indigenous bacteria, and Agrobacterium sp I37 were inoculated in 250 ml of Nutrient Broth (NB) media each. The media was shaken using a shaker at a speed of 55 rpm for 7 days to increase the density of bacterial cells to reach a density of 10¹⁰ cells/ml. To measure the density of bacterial cells using a hemacytometer.

2.4.2. Preparation of inorganic fertilizer. The inorganic fertilizers used were Urea fertilizer at 130.43 kg/ha, SP-36 fertilizer at 55.56 kg/ha and KCl fertilizer at 50 kg/ha [13]. The fertilizer dose is converted according to Biduri’s needs. Dosage for Urea fertilizer on Biduri 0.45 g/pot, SP-36 fertilizer 0.19 g/pot, KCl fertilizer 0.17 g/pot.

2.4.3. Preparation of soil. The soil used for planting media was dried and then filtered using a multilevel sieve and then weighed as much as 7 kg/pot. The next stage is soil sterilization using the steam method (soil is transferred to a baking sheet, and steamed for 2 hours/day, for 3 consecutive days) [14].

2.4.4. Preparation of treatments. The application of indigenous bacterial isolates and Agrobacterium sp I37 was carried out a week before the plants were planted. This is done so that the bacteria can adapt to their new environment. The application dose of bacteria in the soil is 10⁷ per gram of soil. Therefore, the application of bacteria in pots was 7 ml of bacterial inoculum with a density of 10¹⁰ cells/ml. Bacterial inoculum was poured into each planting hole in treatments indigenous bacterial (A1) and Agrobacterium sp I37 (A2). Bacterial application was carried out in pots with doses of Mercury D1 (0.3 μg.g⁻¹), and D2 (5.15 μg.g⁻¹). The dose was then converted according to the weight of the soil, namely D1 of 2.1 mg/pot, and D2 of 36.05 mg/pot.

2.4.5. Planting and harvesting. One pot contains 1 Biduri seed. Maintenance carried out is setting water irrigation, weeding grass, and eradicating pests. Harvesting was done at 90 days. Harvesting by separating the roots and shoots.
2.5. Parameters of laboratory observation and analysis
Parameters observed were initial and final soil characteristics including: soil pH (Potentiometric method), Cation Exchange Capacity (CEC) (Ammonium treatment method), Soil Organic matter (Walkley-Black method), Bacterial population (Plate count method) [15] and Hg levels (using the wet digestion method followed by reading with a mercury analyzer). Parameters observed further were plant characteristics including biomass, and Hg absorption in plants using the wet digestion method followed by reading with a mercury analyzer.

2.6. Data analysis
Data were statistically analyzed using ANOVA (95% significance level) followed by Duncan Multiple Range Test (DMRT) (95% significance level). Correlation test is carried out to see the relationship between the observed parameters.

3. Results and discussion

3.1. Soil characteristics
The initial soil in this study is soil which used for planting media that has been sterilized and has not received any treatment. The initial soil characteristics can be seen in Table 1.

| No | Parameter                          | Value | Unit         | Level          |
|----|------------------------------------|-------|--------------|----------------|
| 1  | Soil pH                            | 6.95  | -            | Netral*        |
| 2  | Cation Exchange Capacity            | 6.13  | cmol(+).kg⁻¹ | Low*           |
| 3  | C Organic                          | 0.08  | %            | Very Low*      |
| 4  | Number of bacterial colonies        | 7.2 × 10² | Log 10 CFU.g⁻¹ |               |
| 5  | Hg Level in Soil                   | 0.13  | µg.g⁻¹       | Below quality standard ** |

Note: *) Level according to the Indonesian Soil Research Institute 2009, **) Level according to Government Regulation no. 101 concerning Management of Hazardous and Toxic Waste (2014). Source: Primary Data (2021)

The characteristics of the initial soil on the planting medium showed that the soil before the bioremediation process had a neutral soil pH of 6.95. A neutral pH value indicates the content of H⁺ ions and the content of OH⁻ ions that can be exchanged in a balanced state C-organic initial soil has a content of 0.083% (very low). This indicates that the soil in the study area has low fertility. Organic matter has an effect on soil pH, cation exchange capacity (CEC) and nutrients in soil CEC soil is relatively low at 6.13 cmol(+).kg⁻¹. The number of bacterial colonies was 7.2 × 10² Log 10 CFU.g⁻¹. The sterilization process using the steam method has not killed bacteria in the initial soil, this is in accordance with Cahyani [14] steam sterilization method is able to kill bacteria about 83.71% higher than the control. The initial soil mercury content is below the quality standard of 0.13 µg.g⁻¹. According to Government Regulation No. 101 concerning Management of Hazardous and Toxic Waste (2014) the threshold value of Mercury in the soil is 0.3 µg.g⁻¹. Heavy metals are absorbed by plant roots in the form of water-soluble ions such as nutrients. The transfer of heavy metals from the soil to plants is carried out by diffusion and osmosis, in which the mass of substances contained in a high medium (soil) will move to a medium with a lower content (plants). Heavy metal absorption is strongly influenced by pH, CEC and organic matter.

3.2. Hg level in final soil
Based on the results of ANOVA, the bioremediation agent, mercury dose, and the interaction between the bioremediation agent and mercury dose significantly affected Hg levels in the soil (p<0.05). Figure 2. shows that the levels of Hg in the soil ranged from 0.06–1 µg.g⁻¹.
Soil sterilization does not eliminate Mercury completely. The initial soil content contained mercury of 0.13 µg.g⁻¹. This causes the dose of mercury to increase so that treatment D1 contains Mercury of 0.43 µg.g⁻¹ while D2 contains Mercury of 5.28 µg.g⁻¹. When compared with the initial soil (Figure 1), Mercury content in the control treatment decreased by 0.09 µg.g⁻¹ or 30.76% higher than the initial soil (before treatment). The decrease in mercury levels is thought to be because the mercury has been absorbed by Biduri. It can be concluded that even without the addition of bacteria Biduri already able to absorb mercury. Based on Figure 1. Treatment without bioremediation agent + dose of mercury 5.15 µg.g⁻¹ (A0D2) was able to reduce Mercury levels in the soil to 1 µg.g⁻¹ or 81.06% when compared to the control whose ability was 30.76%, so the A0D2 treatment was 50.33% higher than the control.

It can be concluded that the Biduri plant without the addition of a bioremediation agent is able to absorb heavy metal Hg. Biduri is also a plant that has a high tolerance for heavy metals because it has the ability to form phytochelatins where peptide compounds produced by plants are able to chelate metals in large quantities. When compared with indigenous bacteria with the same dose of mercury (D2), indigenous bacteria were able to reduce mercury levels in the soil by 0.22 µg.g⁻¹ or 95.83% compared to A0D2. When compared with Agrobacterium sp I37, Agrobacterium sp I37 was able to reduce mercury levels in the soil by 0.26 µg.g⁻¹ or 95.08% compared to A0D2. The given bacteria will be in a symbiotic relationship with plant roots. In this case, plant roots produce root exudates while bacteria will produce secondary metabolites that will transform mercury from inorganic mercury can be converted to toxic methyl mercury [16].

The effectiveness of phytoremediation is the accuracy of the treatment in determining the success of plants absorbing heavy metals which is calculated from the difference in initial and final levels after treatment [17]. The value of the effectiveness of bioremediation can be calculated using the formula:

\[
\text{Bioremediation effectiveness (\%)} = \frac{\text{Initial soil Hg content} - \text{final soil Hg content}}{\text{Initial soil Hg content}} \times 100\%
\]

By using this formula, the calculation results are obtained as shown in Table 2.

Based on Table 2, the effectiveness of phytoremediation by Biduri plants was highest in the treatment of indigenous bacteria + 5.15 µg.g⁻¹ Hg (A1D2) dose of 95.83%. The use of Biduri plants in combination with other treatments has a higher effectiveness than only Biduri plants. The given bacteria will be in a symbiotic relationship with plant roots. In this case, plant roots produce root
exudates while bacteria will produce secondary metabolites that will transform mercury. Even though the Biduri plant is not combined with bacteria, it is also able to absorb Hg with an effectiveness of 30.77%. In addition to the absorption of Hg by plant roots, leaves also absorb Hg from the atmosphere. Therefore, it is inferred that plants can absorb mercury from both the soil and atmosphere [18]. Plants can absorb mercury that is deposited on leaf surfaces. Besides, plants can also uptake mercury from water and soil via roots. Majority of mercury accumulates locally in the plant with little mobility, and only small portions may be released into the atmosphere or transported to other plant organs. Mercury accumulated in plants are in the forms of Hg(0), Hg(II), and organic Hg, and usually aquatic plants contain more methyl mercury (organic Hg) than a terrestrial plant. Mercury can be harmful at very low concentrations because of its high toxicity and ability to bioaccumulate. The mercuric ion is one of the strongest thiol-binding agents, and mercury absorbed into the human body attaches to thiol residues in proteins, making it difficult to eliminate from living organisms. Intracellular mercury can inactivate sulfur, which can inhibit various enzymes, cofactors, and hormones and result in many diseases in animals or humans [19].

### Table 2. Bioremediation effectiveness.

| Treatments | Initial Content (µg.g⁻¹) | Final Content (µg.g⁻¹) | Bioremediation effectiveness (%) | Notes |
|------------|--------------------------|-----------------------|----------------------------------|-------|
| A0D0       | 0.13                     | 0.09                  | 30.77                            | Decrease |
| A0D1       | 0.43                     | 0.39                  | 9.30                             | Decrease |
| A0D2       | 5.28                     | 1                     | 81.06                            | Decrease |
| A1D0       | 0.13                     | 0.06                  | 53.85                            | Decrease |
| A1D1       | 0.43                     | 0.09                  | 79.07                            | Decrease |
| A1D2       | 5.28                     | 0.22                  | 95.83                            | Decrease |
| A2D0       | 0.13                     | 0.23                  | 69.23                            | Increase |
| A2D1       | 0.43                     | 0.48                  | 11.63                            | Increase |
| A2D2       | 5.28                     | 0.26                  | 95.08                            | Decrease |

Notes: A0: Without Bioremediation agent, A1: Indigenous Bacteria, A2: Agrobacterium sp. I37, D0: Without Mercury, D1: Mercury 0.3 µg.g⁻¹, D2: Mercury 5.15 µg.g⁻¹.

According to Hapsari and Lestari [6], Biduri has the ability to absorb Cd at the root of 1.26 µg.g⁻¹ and at the plant crown 1.01 µg.g⁻¹, higher than elephant grass. The level of Cd reduction by biduri plants was 64.76% higher than that of elephant grass. Based on the correlation test, the level of Hg in the soil was negatively correlated and closely related to the dry weight of the Biduri shoots (r=-0.448). The content of Hg in the soil was also positively correlated with the CEC of the soil (r=0.372) but weakly. The increase in soil CEC affects the level of Mercury in the soil. The increase in soil CEC can increase the mobilization of Mercury. Mercury is more easily leached contaminating groundwater, streams, and lakes [20]. The adsorption of Cd was strongly influenced by the competition with other metals and its bioavailability was weakly influenced by ion exchange [21].

### 3.3. Plant characteristics

#### 3.3.1. Plant total dry biomass.

Based on the ANOVA results, the bioremediation agent, mercury dose, and the interaction between the bioremediation agent and mercury dose significantly affected the total dry biomass of Biduri plants (p<0.05). The total dry biomass of Biduri plants ranged from 14.09–73.93 g. The following is a graph of the total dry biomass of plants (Figure 3).

The total biomass of Biduri plants under A1D2 treatment had the highest mean of 73.93 g or 54% higher than the control (Figure 2). According to Ma et al. [22] the presence of microorganisms such as bacteria can dissolve soil nutrients to become available to plants so as to increase plant growth. According to Jumrawati [23] inoculation of Rhizobium sp. produced the highest total dry weight per plant of 7.45 g. The same thing was also reported by Sessitsch et al. [24] inoculation of bacteria Bradyrhizobium sp. 750 in the rhizosphere of Lupinus Luteus plants can increase plant weight by 29%.
Total dry biomass of Biduri was positively correlated and closely related to Hg absorption by Biduri (r=0.967). This means that as the total dry biomass increases, the total absorption of Hg by Biduri will also increase. According to Chen et al. [25] inoculation of bacteria in the rhizosphere of plants growing on Cd contaminated soil significantly increased plant dry weight. Based on Tangahu et al. [26] the success of phytoremediation is characterized by high absorption and accumulation of heavy metals followed by high biomass production [26].

![Figure 3. Total Biomass of Biduri Plants. Notes: A0: Without Bioremediation agent, A1: Indigenous Bacteria, A2: Agrobacterium sp. I37, D0: Without Mercury, D1: Mercury 0.3 µg.g⁻¹, D2: Mercury 5.15 µg.g⁻¹.* Numbers followed by different letters show results that have a significant effect on the DMRT test](image)

3.3.2. Total Hg absorption by Biduri. Based on the ANOVA results, it was shown that the administration of bioremediation agents and the interaction between the bioremediation agents and the administration of mercury doses significantly (p<0.05) increased the total absorption of Mercury by Biduri. The following is a graph of the total absorption of Hg by Biduri (Figure 4).

The total Mercury absorption ranged from 2.11–57.19 µg. The highest total Mercury uptake by Biduri plants in Indigenous Bacteria + Mercury 0.3 µg.g⁻¹ (A1D1) treatment was 57.19 µg or 99.24% higher than the control. Bacterial inoculation in plants has the potential to increase phytoextraction. Microorganisms produce organic extracellular chelators, cidorephores and ligands that can increase the mobility of Mercury so that it can be easily absorbed by plants [24]. Naturally, plants can absorb Mercury through a phytoextraction mechanism. Mercury is often absorbed through nutrient absorption mechanisms. When the mercury content in the soil is high, the plants cannot distinguish between nutrients and mercury due to the load equation. Plants are called hyperaccumulator plants if they are able to absorb mercury more than 10 µg.g⁻¹ [27]. The Biduri plant with the addition of indigenous bacteria was able to absorb 57.19 µg of mercury. It can be concluded that the Biduri plant with the addition of indigenous bacteria is a Mercury hyperaccumulator plant. Plants secrete root exudates consisting of sugar compounds, glycosides, enzymes, vitamins, amino acids, organic acids, nucleotide compounds and their bases, and indole compounds. The content of organic acids in root exudates can reduce soil pH. Mercury compounds and ions will be dissolved so that they are absorbed by plant roots. The adsorbed heavy metals will be translocated and accumulated in stems, roots, leaves, and fruits [28]. High levels of mercury will cause symptoms of poisoning in plants such as stunted plants, chlorosis, and blackening of the root system [29]. Hyperaccumulator plants are plants that can concentrate metals in their biomass in unusually high levels. Based on the correlation test, total Mercury absorption by Biduri plants was positively correlated with total biomass, (r=0.967) so that
the higher the total biomass, the higher the total Mercury absorption by plants. Applying EDTA individually or in combination with bacterial strains (P18 and P15) significantly increased shoot biomass. Application of EDTA in PGPR-inoculated pots increased concentrations of heavy metals in corn shoots and roots compared to the control. The highest concentration of Zn in corn root and shoot was observed in P15 EDTA treatment, which was 2.0-fold and 1.3-fold higher than those in the untreated soil [30].

![Figure 4. Total Hg Absorption by Biduri](image1)

**Figure 4. Total Hg Absorption by Biduri.** Notes: A0: Without Bioremediation agent, A1: Indigenous Bacteria, A2: *Agrobacterium* sp. I37, D0: Without Mercury, D1: Mercury 0.3 µg.g⁻¹, D2: Mercury 5.15 µg.g⁻¹.*) Numbers followed by different letters show results that have a significant effect on the DMRT test.

3.3.3. Effect of bioremediation agents on total Hg absorption by Biduri. The following is the effect of bioremediation agents on the total absorption of Biduri Figure 5.

![Figure 5. Effect of bioremediation agents on total Hg absorption by Biduri](image2)

**Figure 5. Effect of bioremediation agents on total Hg absorption by Biduri.** Notes: A0: Without Bioremediation agent, A1: Indigenous Bacteria, A2: *Agrobacterium* sp. I37.*) Numbers followed by different letters show results that have a significant effect on the DMRT test.

The use of indigenous bacteria was proven to be able to increase the total absorption by Biduri 42.7 µg or 84.73% higher than the control. Inoculation of plants with plant growth promoting microorganisms can protect plants from pathogens and metal poisoning so as to increase the efficiency of phytoremediation [31]. Indigenous bacteria can increase phytoextraction by 85% for As and 45%
for Hg [10]. The application of mercury-resistant bacteria and ammonium thiosulfate increased 157-162% of *P. conjugatum* biomass compared to that without the application of mercury-resistant bacteria. Two isolates of mercury resistant bacteria (*Brevundimonas vesicularis* and *Nitrooccus mobilis*) were applied to *Paspalum conjugatum* as a mercury accumulator plant that was grown for 70 days on gold mine tailing-contaminated soil. The application of mercury-resistant bacteria with ammonium thiosulfate in soil phytoremediation with *P. conjugatum* reduced 18% and 20% Mercury content in the soil contaminated with small-scale gold mine tailings containing mercury. The decrease in mercury content in the soil due to the application of *B. vesicularis* and *N. mobilis* in soil phytoremediation with *P. conjugatum* increased biomass production of a maize plant by 131% and 145%, respectively [32]

4. Conclusion
Mercury content in the control treatment decreased by 0.09 mg/kg or 30.76% higher than the initial soil (before treatment). The decrease in mercury levels is thought to be because the mercury has been absorbed by Biduri. It can be concluded that without the addition of bacteria. Treatment without bioremediation agent + dose of mercury 5.15 mg.kg-1 (A0D2) was able to reduce Mercury levels in the soil to 1 mg/kg or 81.06% when compared to the control whose ability was 30.76%, so the A0D2 treatment was 50.33% higher than the control. It can be concluded that the Biduri plant without the addition of a bioremediation agent is able to absorb heavy metal Hg. Biduri is also a plant that has a high tolerance for heavy metals because it can form phytochelatins where peptide compounds produced by plants are able to chelate metals in large quantities. When compared with indigenous bacteria with the same dose of mercury (D2), indigenous bacteria were able to reduce mercury levels in the soil by 0.22 mg/kg or 95.83% compared to A0D2. When compared with *Agrobacterium* sp I37, *Agrobacterium* sp I37 was able to reduce mercury levels in the soil by 0.26 mg/kg or 95.08% compared to A0D2.

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