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Effect of *Azotobacter* sp. inoculation on sweet potatoes (*Ipomoea batatas* L.) yield in lead-contaminated soil

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Abstract

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The presence of Pb in the soil has negative effects not only on soil health but also on the growth and yield of food crops. *Azotobacter* is a beneficial microbe that has the ability to reduce the availability of heavy metals in soil. This study aimed to determine *Azotobacter* susceptibility to Pb and their effect on the growth and yield of sweet potato grown in lead-contaminated potted soil. The susceptibility test was performed in Pb-contaminated Ashby broth. The pot experiment was carried out in a factorial randomized block design. The first factor was the dose of *Azotobacter* usk1 liquid inoculant (0, 10, 20, and 30 mL/pot) while the second one was the Pb contamination level (0, 100, and 200 mg/kg). The results showed that *Azotobacter* usk1 was resistant to 0.1 mg/kg Pb. Inoculation of 20 mL/pot *Azotobacter* usk1 increased sweet potatoes growth and yield in soil contaminated with 10-30 mg/kg Pb.

Keywords: Azotobacter sp, *Ipomoea batatas* L.; lead contamination

Introduction

Sweet potato (*Ipomea batatas* L.) is one of the staple foods that has been cultivated in Indonesia. Tubers contain protein, carbohydrates, minerals (calcium, iron, sodium and potassium), carotenoids, dietary fibre, vitamins (especially C, folate, and B6), and a very low quantity of fat (Reddy et al., 2005; Sun et al., 2014). Sweet potatoes are the promising source of nutrition to substitute other staple food mainly rice. In the tropics, sweet potatoes are usually grown in extended post-rice lowland areas where the soil has a mid to low source of nutrients (Loebenstein & Thottapilly, 2009). The occurrence of changes in agroecosystems from dry land to paddy fields led to agronomic changes in sweet potato cultivar, which were shown by the characteristics of storage root weight and fresh vines weight per plant and estimated yield per hectare and root/shoot ratio. Sweet potato is a plant that is easy to adapt to the conditions of various agroecosystems, although the response of these plants generally varies when cultivated in different agroecosystems, due to the interaction between genotypes and the environment.

Lead is often found in soil and in the form of galena (PbS), anglesite ($PbSO_4$), or cerussites ($PbCO_3$) and cation (Pb^+). The normal threshold of lead in soil is 2 – 300 ppm while the critical limit is 100 – 400 ppm (Ahmed & Tajmir-Riahi, 1993). However, the threshold is varying in each country. In Finland, the normal threshold value is 60 ppm (Gothberg, 2008) while in India is 250-500 ppm (Ali & Nas, 2018). The limit of Pb in residential and agricultural areas is 400 ppm in Thailand (National Environment Board, Ministry of Natural Resources and Environment, 2004).

Human activity in the lowland might affect the quality of the surrounding agricultural area including increase heavy metals content. Improper wastewater treatment from the industrial area that dissipated to the planting area is reported to enhance the metal content (Dar et al., 2015). The presence

of metal contaminants in the soil is a constraint to achieve the optimal growth and yield of tuberous plants. Most of the lead absorbed (approximately 95% or more) is accumulated in the roots, and only a small fraction is moved to the upper plant parts (Purrout, 2011). It has been reported that lead was found in the root and leave parts of sweet potato (*Ipomea batatas* L.) planted in Nigeria with pollution indices of 0.4 and 0.13 (Wilberforce, 2013). The increase of Pb in the tuber reduced the tuber quality and menace human health.

High Pb content in the plant inhibits the growth because it inhibits two enzymes namely aminolevulinic acid and porphobilinogen which are involved in chlorophyll biogenesis (Bovell-Benjamin, 2007). Lead also changes the lipid composition of thylakoid membranes contain chlorophyll (Stevanov, 1995) and harms the photosynthetic apparatus due to its affinity for protein N-and S-ligands (Ahmed et al., 1993; Ali, 2018). Sequentially, lead limited the uptake of essential elements such as magnesium and iron by plants. Lead contamination is not only restricting the growth and yield of plants but also changes the quality of tubers due to the accumulation in the tubers that treated food security.

One of the solutions to combat this phenomenon is bioremediation. Bioremediation is one method that can be applied to accelerate the reduction of heavy metal toxicity to the soil by using organisms include plants and microbes. Algae, fungi or bacteria are used as adsorbents for metal ion absorption (ref). Microorganisms absorb heavy metals actively within living cells (bioaccumulation) or passively on the surface of dead or living cells through biosorption (Cenkci et al., 2010). Lead biosorption by microbes is to carry out by ionic binding between positive-charged metal ions and negatively charged ions in cell walls or extracellular polymers known as exopolysaccharides (EPS) (Luqman et al., 2012). The negative charge in the cell surface and EPS originated from a functional group of carbonyls, carboxyl, amino, ketone and hydroxyl (Frederick et al., 2011).

One of the microorganisms that can be involved in the biosorption process is Azotobacter. The genus of *Azotobacter* is a Plant Growth Promoting Rhizobacteria (PGPR) live in the rhizosphere. They induce plant growth by fixing the dinitrogen and releasing phytohormones. Researchers reported that Azotobacter is relatively resistant to heavy metals Pb (Miller & Bassler, 2001. *Azotobacter* is also able to produce exopolysaccharides (EPS) which play an important role in the process of metal biosorption (Gauri et al., 2012). Exopolysaccharides can adsorb heavy metals such as Pb because EPS is negatively charged and the metal forms ligand bonds with EPS (Khan et al., 2015). The EPS of Azotobacter enable to adsorb heavy metal has been recorded (Rasulov et al., 2013)

It has been stated that soil inoculation with rhizobacteria as PGPR can result in lead immobilization within the rhizosphere soil through the arrangement of chelate compounds and complex compounds with Fe hydroxides (Belogolova et al., 2020). Hadi & Bano (2010) concluded that diazotrophic microbes (*Rhizobium* and *Azotobacter*) increased maize growth on heavy metal polluted soil to reduction in metal toxicity. Belogolova et al. (2020) also reported a decrease in lead uptake by upper parts of wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), pea (*Pisum sativum* L.) and radish (*Raphanus sativus* L.). Microbial inoculation in the soil is expected to improve plant growth and yield in lead-contaminated soil. The objective of this research was to verify the resistance of Azotobacter on Pb and the effect of Azotobacter inoculation on the growth and yield of sweet potatoes grown in soil with several levels of lead concentration.

Materials and Method

The two-steps experiment has been carried out from March until September 2019. Laboratory experiment for susceptibility test on lead was performed in the laboratory of Soil Biology Faculty of Agriculture Unpad Sumedang, while pot experiment for assessing the effect of Azotobacter inoculation on sweet potatoes yield was done in experimental field Faculty of Agriculture Unsika Karawang. The experimental field is located in the tropics at the altitude of 18.2 m above sea level.

The Azotobacter usk1 was isolated from the saline paddy field in Tempuran, Karawang, West Java. The cell morphology of Gram-negative Azotobacter was mono or diplococcus (Figure 1). The nitrogen fixation of this isolate was 0.0026 µmol/hour based on Acetylene Reduction Assay. These bacteria secreted 41.33 g/L of EPS, 2.23 ppm of IAA, as well as 1.42 ppm of GA, 0.49 and 0.57 of phytohormones zeatin and kinetin respectively.

The soil for the pot experiment has been taken from saline paddy field located in Tempuran, by using N-free Ashby's Medium. The paddy field's soil is Inceptisols which is rich in macronutrients N, P and K and has neutral acidity (Table 1). Before the experiment, the soil was air-dried without direct sunlight for 3 days.

Lead susceptibility test

The susceptibility of *Azotobacter* usk1 on lead was tested on Ashby's broth (mannitol 10.0 g; K_2HPO_4 0.5 g; $MgSO_4 7H_2O$ 0.2 g; NaC1 0.2 g; $CaCO_3$ 0.1 g; $NaNO_3$ 0.1 g; $FeSO_4$ 0.1 g; Na_2MoO_4 0.01 g; distilled water 1000 mL) contaminated with lead. The contaminant levels were 1, 10, and 100 mg/kg $Pb(NO_3)_2$. The control treatment was Ashby's broth

Table 1. Chemicals characteristic of the soil used in this study

Chemical characteristic	Score	Unit	Category
pH H_2O	7.3	–	Neutral
P_2O_5 (Olsen)	238	–	Very high
P_2O_5 (HCl 25%)	0.128	Ppm	Very high
K_2O (HCl 25%)	0.042	%	High
N Total (Kjeldahl)	0.26	%	Mid
C Organic (Walkey&Black)	3.2	%	High
C to N ratio	12.3	%	Mid

Source: Indonesian Center of Biodiversity and Biotechnology Laboratory, Bogor, West Java

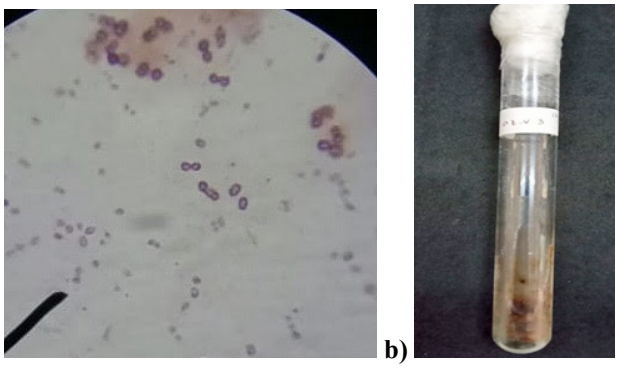

Fig. 1. Cell Morphology of Gram-negative *Azotobacter* usk1 (*a*) and their pigmentation in N-free slant (*b*)

without Pb. In the preliminary study, Azotobacter usk1 enabled to grow in the N-free Ashby broth contaminated with 1 mg/l $Pb(NO_3)_2$. All treatments were replicated three times. A total of 50 mL of Ashby's broth with and without Pb were poured into 100 mL Erlenmeyer, autoclaved for 20 min at 121°C and left overnight at room temperature. All growth media were inoculated with 1% of Azotobacter liquid cultures, incubated 72 hours prior to count the Azotobacter population by using the serial dilution plate method (Sanders et al., 2012). The liquid inoculant was diluted in NaCl 0.85%. A total of 1 mL of Azotobacter suspension from 10^5 dilutions was placed in the Petri dish and mixed with 20 mL of Ashby's Agar at 40°C. Azotobacter colonies in plate agar were counted after 72-day incubation at 30°C.

Experimental establishment

The experiment was carried out in factorial randomized block design with inoculation dose of *Azotobacter* usk1 as the first factor consisted of without inoculation (control), 10 mL/pot, 20 mL/pot and 30 mL/pot. The second factor was the level of Pb contamination consisted of without contamination (control), 100 mg/kg $Pb(NO_3)_2$, and 200 mg/kg $Pb(NO_3)_2$. All treatments were then replicated 3 three times.

The potting soil was mixed with 25% manure based on the total weight of the soil. A 10 kg of mixed soil and manure were put in a polyethylene bag (30 cm x 50 cm). The soil was contaminated with $Pb(NO_3)_2$ according to the level described above before placed in the bags. The $Pb(NO_3)_2$ was diluted in water, poured on potting soil and incubated for 14 days. The 20-cm stem cuttings of local sweet potato cultivar Rancing were grown in a pot and placed in the field without shade for 60 days.

One loop of Azotobacter pure culture was grown in sterilized 100 mL NB broth in 250 ml Erlenmeyer flask for 7 days on a gyratory shaker at 115 rpm. After 7 days of incubation, Azotobacter liquid inoculant was mixed with 90 mL of water and applied to the soil in the pot before planting. The Azotobacter population of the liquid inoculant was about 10^6 CFU/ml. The volume of the Azotobacter inoculant was dependent on the treatments described above. Three seedlings of sweet potatoes were grown for 7 days when a single best seedling was maintained until the harvest time at 90 days after planting (dap). The recommended inorganic fertilizers included Urea 200 kg/ha; SP36 100 kg/ha and KCl 100 kg/ha were applied in a single dose at 30 days after planting.

Parameters and statistical analysis

At the late vegetative stage, 49 days after planting, the length of tendril was measured and a number of leaves were counted. The chlorophyll content of mature leaves was measured by SPAD. The yield characters were observed at 90 daps included the number, weight, length, and diameter of the tuber. The tubers were stored for 28 days at room temperature (23-35°C) without direct sunlight. The sweetness level (°brix) of the yield was measured on the 7th, 14th, and 28th days after storing. All of the data were subjected to analysis of variance and Duncan multiple range test with $p < 0.05$ by using IBM SPSS 25 software.

Results

Azotobacter Susceptibility to Lead

The growth of Azotobacter in Ashby broth depends on the level of Pb. Contaminating broth with 1 mg/L enhanced Azotobacter count significantly compared to the control (Figure 2). None the less high level of Pb reduced the population by almost two logs during 72-day incubation.

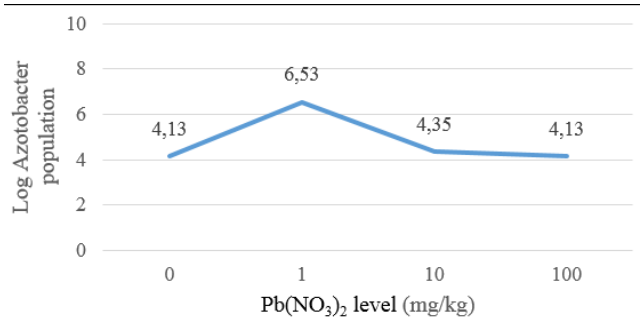

Fig. 2. Log of Azotobacter population in contaminated media with Pb

Vegetatif growth of sweet potato

The analysis variance showed that there was no significant interaction effect between the level of Azotobacter and Pb on the tendril length of sweet potato. Independently, both treatments didn't influence tendril length. Azotobacter inoculation mostly tended to increase tendril length but adding 30 ml liquid inoculant decreased tendril length at 21-49 dap (Figure 3). Regardless of statistical analysis, higher tendril length was evidence in soil without Pb compared to soil with Pb at days 21 (Figure 3a) and 49 (Figure 3c). The lowest tendril length decreased due to 30-ml Azotobacter inoculation was recorded at day 49 compared with days 21 and 35. On day 49: the tendril length with 30-ml bacterial inoculation with 200 mg/kg Pb was 9.5% less than the plant with 100 mg/kg Pb (Figure 3c).

The analysis of variance showed that the effect of Azotobacter on leaves number didn't depend on the level of Pb contamination. Regardless of statistical analysis, plant grown in soil with 200 mg/kg Pb tend to have the lowest leaves number compared to that with 100 mg/kg Pb and control (Figure 4). In common, Azotobacter inoculation up to 20 ml per pot had a tendency to increase leaves number. In line with the length of the tendrils, the leaves number also decreased at 30 ml of Azotobacter inoculation. The Figures 3 and 4 indicate that plants enable to grow well in lead-contaminated soil when accompanied with 20 mm of Azotobacter inoculation.

Based on analysis variance, the effect of Azotobacter inoculation on chlorophyll level didn't depend on the contamination of Pb level. The Azotobacter inoculation has a potency to increase the chlorophyll although statistically was not significantly different from the control. Increased Pb level in the soil also did not change chlorophyll content. Regardless of statistical analysis, inoculation of 30-mL Azotobacter inoculation in any Pb level was likely to cause a higher chlorophyll value compared to the lower dose of Azotobacter. The Highest Pb contamination re-

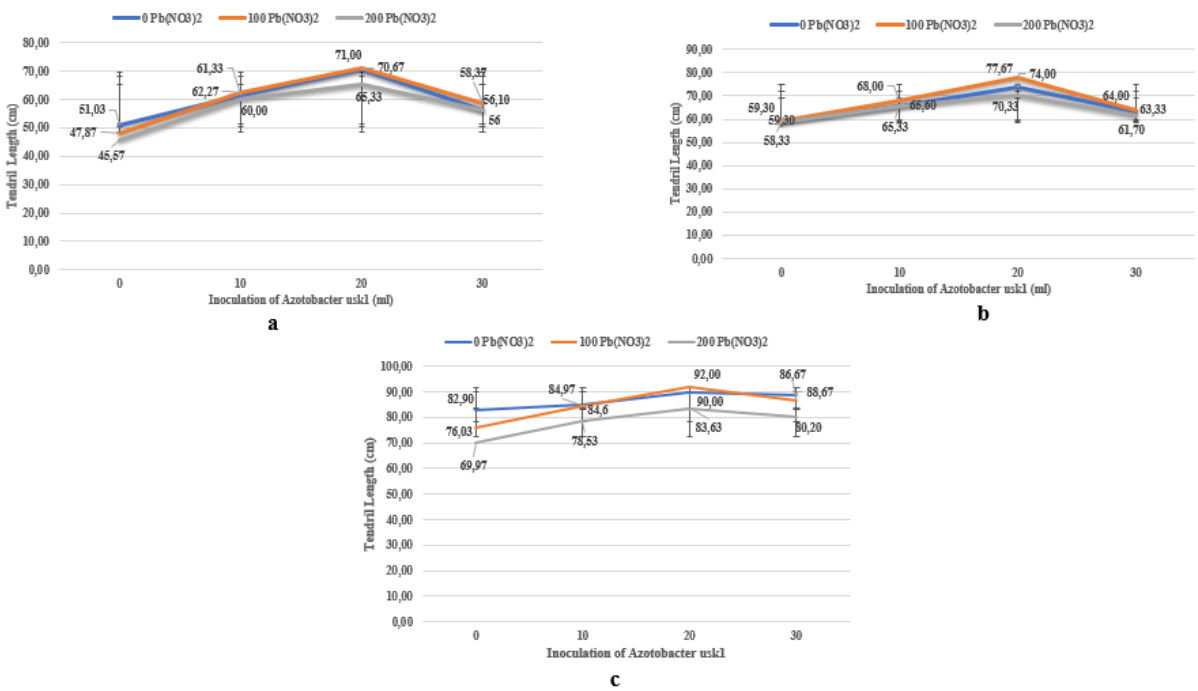

Fig. 3. Tendril length of sweet potatoes grown in soil with some level of Pb contamination and Azotobacter inoculation on 21 days after planting (*a*), 35 days after planting (*b*), and 49 days after planting (*c*)

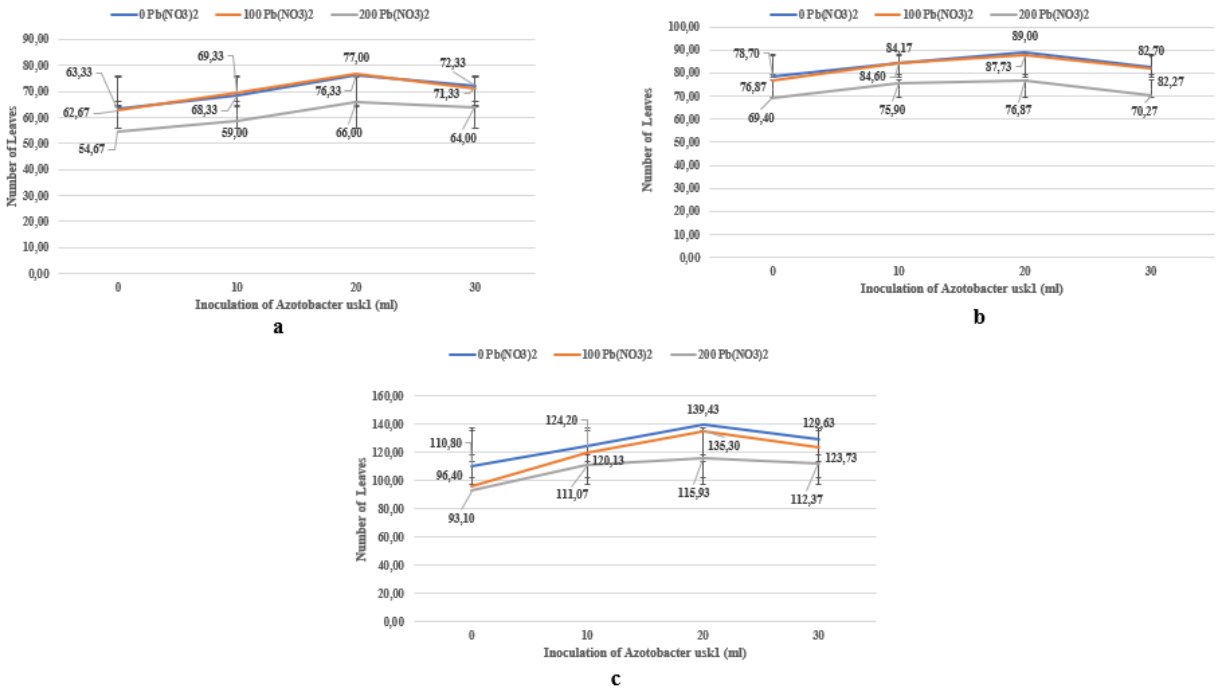

Fig. 4. Leaves number of sweet potatoes grown in soil with some level of Pb contamination and Azotobacter inoculation on 21 days after planting (a), 35 days after planting (b), and 49 days after planting (c)

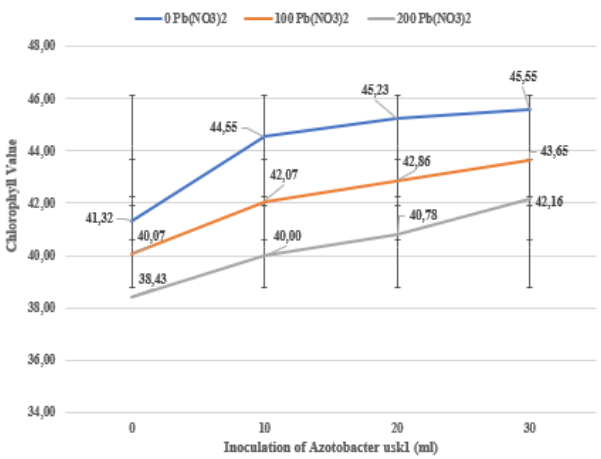

Fig. 5. Chlorophyll content on mature leaves of Sweet potatoes grown in several levels of Pb contamination and Azotobcter inoculation at 45 days after planting

sulted in the lowest chlorophyll value for each level of Azotobacter. This indicated that the use of *Azotobacter* usk1 increased the value of chlorophyll even though the soil was contaminated with Pb).

Total Lead in Soil

Based on the Anova, the level of Azotobacter inoculation and Pb contamination has a significant interaction effect on the amount of Pb in the soil at 45 days after planting (Figure 5). In soil with 100 and 200 mg/kg Pb, 20 mL of Azotobacter inoculation decreased total Pb in soil compared to the soil with 10 ml inoculant and control (Table 2.). The Pb in soil with 100 mg/kg Pb was higher than Pb in contaminated with 200 mg/kg Pb when Azotobacter has not inoculated or inoculated with 10 ml. In soil with 30 ml Azotobacter inoculation, total Pb in soil with 200 mg/kg Pb was lower higher than Pb in soil with lower Pb.

Yield parameters

The Anova showed that there was no significant interaction effect between Azotobacter inoculation and Pb level on the yield parameter of sweet potato (Table 3). Independent on lead contamination, Azotobacter affected tuber number, weight and diameter but did not change tuber length. The effect of Pb was only significant on tuber number. Consistently, 20 ml of Azotobacter inoculation gave the highest tuber number and height but was not significantly different in tuber length and diameter. Plants without bacterial inoculation showed the lowest number, weight, length and diameter of tubers. Contamination of Pb only affects the number of tubers; the decrease of that

trait only showed by plants grown in soil with 200 mg/kg Pb, 100 mg/kg Pb and control treatment.

The interaction effect between the Azotobacter inoculation and the level of Pb contamination level on the total Pb in the tubers was significant (Table 4). Any dose of Azotobater inoculation decreased Pb content in tuber grown in soil with 100 and 200 mg/kg Pb. Moreover, 200 mg/kg Pb increased Pb in tuber at any dose of Azotobacter inoculation included control treatment. Azotobacter inoculation ikely enabled to increase in Pb in the tuber.

Table 2. Total of lead in soil at 45 days after planting of sweet potato by Azotobacter inoculation in Pb contamination soil

Pb level, mg/kg	*Azotobacter* usk1 inoculation, ml			
	Control	10	20	30
	mg/kg			
Control	0.56 a A	0.60 a A	0.55 a A	0.59 a A
100	1.03 a B	0.97 a B	0.75 a A	0.77 a B
200	1.14 b B	1.00 b B	0.61 a A	0.73 a AB

Note: The number followed by the same letter was not significantly different according to Duncan's Multiple Range Test at the 5% level. Lowercase letters are read vertically and capital letters horizontally

Table 3. Yield component of sweet potato by Azotobacter inoculation in Pb contaminated soil

Treatment	Yield Component			
	Tuber number	Tuber weight, kg	Tuber length, cm	Tuber diameter, cm
Azotobacter *i*noculation, ml				
Control	2.44 a	0.97 a	11.41 a	3.67 a
10	3.44 ab	1.65 ab	14.25 a	6.07 ab
20	4.89 c	2.64 c	16.04 a	8.45 b
30	4.00 bc	2.18 bc	15.01 a	6.86 ab
Pb, mg/kg				
Control	4.33 b	2.05 a	14.01 a	6.16 a
100	4.25 b	2.04 a	14.77 a	7.02 a
200	2.50 a	1.49 a	13.75 a	5.62 a

Note: The number followed by the same letter was not significantly different according to Duncan's Multiple Range Test at the 5% level

Table 4. Lead content in the tuber of sweet potato by Azotobacter inoculation in Pb contamination soil

Pb level, mg/kg	Azotobacter inoculation, ml			
	0	10	20	30
	mg/kg			
Control	A A	0.41 b A	0.27 ab A	0.32 b A
100	0.57 a B	0.51 a A	0.47 a A	0.49 a A
200	0.67 b B	0.54 ab A	0.35 a A	0.41 a A

Note: The number followed by the same letter was not significantly different according to Duncan's Multiple Range Test at the 5% level. Lowercase letters are read vertically and capital letters horizontally

Sweetness level of tuber

The results showed that there was no interaction effect between *Azotobacter* inoculation and the level of Pb contamination on the sweetness of the tubers at 0 days of observation, 14 days after storage and 28 days after storage. The sweetness level of the tubers is quite fluctuating, measurements at harvest showed the lowest average value when compared to 14 and 28 days after storage. The highest average sweetness level (11.67° brix) at harvest was found in the treatment of 100 mg/kg Pb with 10 ml of *Azotobacter* but at 14 days after storage, the same treatment gave the lowest level of sweetness, namely 12.33° brix. In general, the sweetness level increased 14 days after storage but decreased 28 days after storage. The highest level of sweetness at 28 days after storage was found in the treatment of 20 ml of *Azotobacter* without Pb contamination of 14.00° brix (Table 5).

Table 5. The tuber sweetness level of sweet potato Azotobacter inoculation in lead contamination soil

Treatment	Days of storage		
	0	14	28
	°brix		
0 ml Azotobacter (control) + 0 mg/kg Pb	10.67	14.67	13.00
0 ml Azotobacter + 100 mg/kg Pb	9.33	14.67	10.67
0 ml Azotobacter + 200 mg/kg Pb	9.67	15.33	13.33
10 ml Azotobacter + 0 mg/kg Pb	10.00	13.67	11.67
10 ml Azotobacter + 100 mg/kg Pb	11.67	12.33	12.00
10 ml Azotobacter + 200 mg/kg Pb	10.33	12.67	10.33
20 ml Azotobacter + 0 mg/kg Pb	10.00	12.33	14.00
20 ml Azotobacter + 100 mg/kg Pb	9.67	12.00	11.67
20 ml Azotobacter + 200 mg/kg Pb	10.00	12.33	11.67
30 ml Azotobacter + 0 mg/kg Pb	10.67	14.33	12.33
30 ml Azotobacter + 100 mg/kg Pb	10.00	13.67	12.00
30 ml Azotobacter + 200 mg/kg Pb	10.67	14.00	12.00

Discussion

Azotobacter populations increased in liquid media with 1 mg/kg Pb contamination and could still survive when contamination level is increased up to 100 mg/kg Pb. Nonetheless, the population decreased compared to bacterial count in media with 1 mg/kg Pb. This proved that Azotobacter had the ability to grow in Pb-stressed conditions. The well-known mechanism by which Azotobacter avoid heavy metal poisoning is producing exopolysaccharides (Rizvi et al., 2019). Hindersah et al. (2009) explained that the Azotobacter population increased related to the increase in lead nitrate levels in liquid culture.

Azotobacter resistance to heavy metals is caused by metallothionein protein which sequesters heavy metals in microbial cells (Robinson et al., 2001). Metallothionein sequestrates heavy metals in vacuoles so that heavy metals do not enter Azotobacter's metabolic system so that they are not harmful to their metabolism. The content of metallothionein in Azotobacter cells was induced by Cd, Cr and nickel which explained the close relationship between metallothionein and exogenous heavy metal exposure (Rizvi et al., 2019).

In this study, tendril length and the number of sweet potato leaves in plants with Azotobacter inoculation of 20 ml/pot was higher than the control. Azotobacter is able to increase plant growth through nitrogen fixation (Tilak et al., 2005), and the production of phytohormones (Vikhe, 2014) as well as exopolysaccharides (Gauri et al., 2012; Hindersah et al., 2018). The increase in growth traits by Azotobacter might be related to two mechanisms: acquisition of available nitrogen resulting from nitrogen fixation and increasing levels of plant phytohormones derived from the phytohormones produced by Azotobacter. This mechanism can directly increase N uptake and plant roots that induce vegetative growth. The production of EPS by Azotobacter can also protect plants from the negative impact of heavy metals. The positive effect of Azotobacter usk1 inoculation on the growth of sweet potato growth in Pb-contaminated soil proved Azotobacter resistance to lead and a positive impact on the vegetative growth of plants. The result of the research agrees with the resistance of 17 Azotobacter isolates in the field was quite strong against Pb and Cr (Narula et al., 2012).

Irrespective of Azotobacter inoculation, higher Pb content in growing media reduced the length of tendril, leaves number, chlorophyll value and total Pb content in soil and tuber. This study confirmed that lead affects tendril growth by inhibiting the elongation of the tendril. The highest tendril of 20 ml Azotobacter and 100 mg/kg Pb might associate with the application of *Azotobacter*. The inoculation of *Azotobacter* in plants affects the biosorption mechanism of metals and facilitates the plant in nutrition fulfilment (Kukreja et al., 2004).

Leaf chlorophyll was not affected by Azotobacter inoculation or the level of Pb contamination; however, the results showed that Azotobacter had the potency to increase chlorophyll. Plants inoculated with Azotobacter, enable uptake of more N due to N availability increment in soil. Rueda et al. (2016) explained that the use of biofertilizers containing *Azotobacter* sp increased the amount of leaf chlorophyll by 53.37 ccl compared to control on strawberry plants in the hydroponic system.

Pb accumulation in soils is caused by the increase of Pb levels in the soil. Pb contamination is reported to inhibit the synthesis of leaf chlorophyll (Rueda et al., 2016). Our experiment showed that the value of chlorophyll measured by SPAD was decreased due to high Pb contamination in soil. Piotrowska et al. (2009) showed that *Wolffia arrhiza* grown in Pb contaminated soil had chlorosis symptoms in the leaves due to loss of chlorophyll during 2 weeks. The inhibition mechanism on the chlorophyll biosynthesis is caused by Pb deactivation on enzymes involved in the chlorophyll biosynthesis. Lead contamination prevents some nutrients uptake, such as Mg and Fe and also delays the regulation of ALA (δ-Aminolevulinic acid) and ALAD (δ-aminolevulinic acid dehydratase) activity as the key compound of chlorophyll formation (Aweng et al., 2011). Application of *Azotobacter* could reduce the negative effect of this contamination by EPS synthesis mechanisms. In general, the bacteria enable to sequestrate metal extracelullar (Durand et al., 2015) i.e by EPS. Sequestration by EPS outside the cell wall has been demonstrated by EPS A. chroococcum XU1 for Pb and Hg up to 40-47% of the metal present in the solution (Rasulov et al., 2013). The functional groups such as thiol, amino, carboxyl and also phenolic groups in the surface of EPS helps the immobilization of heavy metal ions in the polluted area (Dar et al., 2015). When the EPS producer bacteria were inoculated, it binds the heavy metals and forms the bond.

Lead is toxic to plants even though present as a trace element in soil (Mahapatra et al., 2020). Reducing the toxicity of Pb is important to stabilize or immobile the metal in order to prevent the uptake of plants. It has been reported that *Azotobacter* has EPS that is tolerant to drought and other obstacles including heavy metals. EPS production is regulated by quorum sensing (the regulation of gene expression in response to fluctuations in cell density) in the early development stage (Gupta, 2016). Quorum sensing regulates some factors of EPS producing bacteria such as channel production within the biofilm, swarming activity, and lipid production (Loebenstein & Thottapilly, 2009). The Pb sequestration by Azotobacter's EPS might help plants to grow with less constraint of metal toxicity.

Conclusions

Azotobacter usk1 has the ability to grow even in stress conditions of up to 100 mg/kg Pb with the mechanism of forming exopolysaccharides. The inoculation of Azotobacter in sweet potato growing media contaminated with Pb has the potential to increase tendril length, leaf number and leaf chlorophyll compared without Azotobacter inoculation. Azotobacter usk1 was able to increase the photosynthate rate of plants and was increasing the yield of sweet potato plants. Contamination of Pb in the soil decreased plant performance which represented in decreasing of the length of the tendrils, the number of leaves and chlorophyll. The increase in Pb accumulation was in line with the increase in the level of Pb contamination in the soil which had a negative effect on tuber yields.

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