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Research article

Implications of Leather Tanning Wastewater Exposure for Soil Bacteria Viability and Phosphate Solubilizing Activity

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Abstract.
Leather tannery wastewater pollutes the environment because it contains BOD, COD, and chromium at levels above safe thresholds. The main objective of this study was to determine the effect of exposure to tannery wastewater on the viability of the soil PSB community and selected PSB isolates, and to examine the ability of selected bacterial isolates to dissolve phosphate in Picovskaya's broth medium supplemented with tannery wastewater. The viability of the bacterial community was determined based on their growth in soil exposed to the waste at concentrations of 30, 60 and 100%, under field capacity moisture conditions, while the ability of the isolates to dissolve phosphate was observed using liquid Pikovskaya's medium which was added to the waste so that the concentration reached 30, 60, and 100%. The bacterial isolates RP-1 and RP-2 were used, which were obtained from the soil surrounding the tannery which was contaminated with leather tanning waste. The parameters that were analyzed were the number of cells and the amount of soluble phosphate. The number of cells was determined through the pourplate method using an agar nutrient medium, and the amount of soluble phoshate was examined using the P chlorostannous reduced molybdophosphoric acid blue method. According to the findings of this study, exposure to effluents reduced PSB viability in the soil. Exposure to waste also negatively affected cell viability and the ability of isolates to solubilize phosphate.

Keywords: Wastewater, tannery, viability, ability, phosphate, solubilization

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1. Introduction

Leather tannery industry is one of the main causes of water pollution. In general, the tanning process consists of 3 basic sub-processes: preparation, tanning, and crusting stages. Pre-treatment of tanning was conducted preservations, soaking, liming, unhair-ring, fleshing, splitting, bating, degreasing, and pickling. anning is the process by which the protein in rawhide is converted into a stable material. Crusting consists of phases such as dilution, retanning, smearing, and coloring. [1]. This industry consumes a lot of water and uses a number of chemical compounds such as Ca(OH)2, Na2(SO3), (NH4)2SO4, NaCl, tannins, and chrome salts [2]. The character of leather tanning waste

has a high pH and contains several specific pollutants such as sulfite, lime and chromium compounds [3], [4]. Chromiun (III) compound are used most often as tanning agent. Released pollutants, together with heavy metals have a negative impact at surrounding lead toxicity to organism. In addition to inorganic pollutants, tanning waste leather contains high organic pollutants, derived from meat and fat and hair attached to the skin. This resulted in the tannery waste having high BOD and COD values far exceeding the predetermined threshold so that it could pollute the environment [3],[4]

Exposure to tannery waste results in changes in physical and chemical properties that can directly or indirectly affect the population, activity and viability of soil microbes. External factors such as high pH, heavy metals level, and toxic compounds greatly affect the activity and viability of bacterial cells [2]. Exposure to tannery waste results in changes in physical and chemical properties that can directly or indirectly affect the population, activity and viability of soil microbes. External factors like high pH, heavy metals level and toxic compounds greatly affect the activity and viability of bacterial cells [2]. Observations on viability describe the ability of microbes to survive in an environment that does not support their growth.

Phosphate solubilizing bacteria (PSB) play an essential role within the biogeochemical process of phosphate cycle. Phosphate compounds are involved in the plant main metabolic processes such as photosynthesis, macromolecular biosynthesis, energy transfer, signal transduction, and respiration [5]. However, phosphate is usually present in an insoluble form in the soil so that it cannot be utilized by plants. PSB has the ability to dissolve phosphate minerals through the mechanism of phosphate anion exchange by acid anions, or the chelation of Al, Ca and Fe ions associated with P by acid anions [6].

Several researchers have shown that PSB have been isolated from land contaminated with leather tanning industry waste [7] – [11]. However, assays on the viability and phosphate solubilizing activity of PSB in tannery wastewater have not been widely disclosed. This assay needs to be carried out to ensure that the bacteria to be applied still have the character of being able to dissolve P even in environmental conditions contaminated with waste. This research was conducted to determine the effect of exposure to tannery wastewater on the viability of the soil PSB community and the selected PSB isolates, and to determine the selected bacterial isolates ability to solubilize phosphate in Picovskaya's broth medium supplemented with tannery waste water.

2. Methodology

Protocol of research stages has been shown in Figure 2.

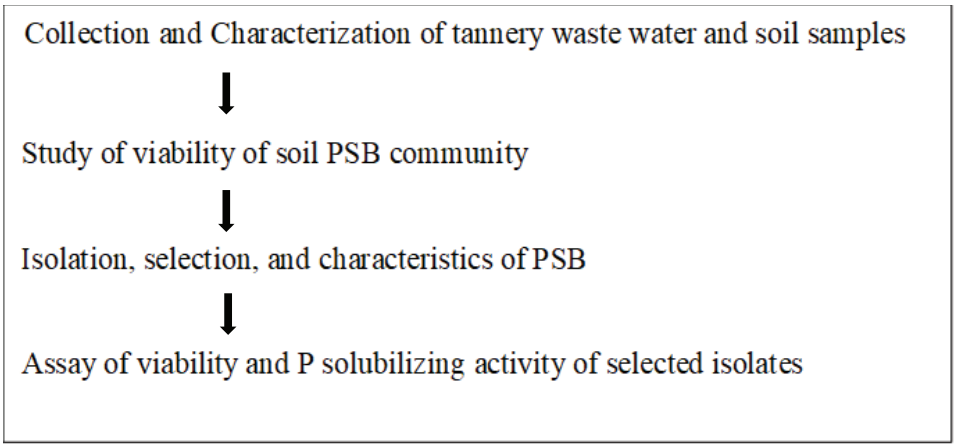

Figure 1: Process of research stages.

2.1. Collection and characterization of tannery waste water and soil samples

Tannery wastewater samples were collected in pre-sterilized screw cap bottles from a leather tanning factory in Magelang, Indonesia especially the final drainage effluent. Then, the samples were transported to the laboratory and were preserved at 4˚C. pH were tested immediately using pH meter. Chromium in samples was removed using conc. HNO_3 and measured by colorimetric method using spectrophotometer at 357.9 nm wave length [12]. BOD and COD B were determined by 5-dDay BOD test and Closed Reflux, Titrimetric Method, respectively. Soil samples both with and without effluents were collected from the surrounding areas of the tannery industries. These two soil samples were air dried and shifted to <2 mm sieves for determination of soil chemical properties (C organic content and Cation Exchange Capacity/CEC) were determined in accordance with standard analytical methods .

Study of viability of soil PSB community exposed to the waste at waste concentrations of 0, 30, 60, and 100%. The viability of soil Phosphate Solubilizing Bacteria (PSB) community was determined based on their growth on the soil supplemented with tannery waste at concentrations of 0, 30, 60, or 100%, under field capacity moisture conditions. Each sample soil that has been treated with waste exposure was incubated at 37ºC for 20 days. The number of PSB was observed after incubation period of 0, 10 and 20 days. For PSB enumeration, one gram of soil from each treatment was

diluted and injected amount 0.1 ml into Pikoskaya's Agar medium consisting: tri-calcium phosphate (TCP) 5 g; potassium chloride 0.2 g; yeast extract 0.5 g; ammonium sulphate 0.5 g; glucose 10 g; sodium chloride 0.2 g; ferrous sulphate trace; magnesium sulphate 0.1 g; agar agar 15 g; manganese sulphate trace; distilled water 1 L; the pH was adjusted to 7.0±0.2 then sterilized in autoklaf [13]. The petridish were stored for 4 days at room temperature. Colonies formed then observed after incubation that showing clear zone around and counted the diameter.

2.2. Isolation, selection, and characteristics of PSB

Discrete colony as a result of the above assay that showing halo zones were isolated and selected based on Phosphate Solubilization Index (PSI) and colony morphology such as color, form, elevation, margin and surface. PSI was calculated by using the formula: the total diameter ratio (colony + halo zone) and the colony diameter [13]. The isolates with the highest and lowest Phosphate Solubilization Index were used for further research.

2.3. Assay of cell viability and P solubilizing activity of selected isolates.

Observations of viability and P solubilizing activity were carried out on Picovskaya's broth medium supplemented with tannery waste water at concentrations of 0, 30, 60, or 100%.

Cell viability: Each of the two selected isolates culture were grown on Pikovskaya Broth media supplemented with 0%, 30%, 60%, or 100% waste and incubated by rotary shaker at room temperature for 20 days at 180 rpm. The viability of soil PSB isolates was determined based on their growth in Pikovskaya's media broth. The number of PSB isolates was observed after incubation period of 0, 10 and 20 days. Cell density was determined, one ml of isolate culture was serially diluted and 0.1 ml was injected into Nutrient Agar medium incubated at room temperature for 48 hr.

P solubilizing activity: Phosphate solubilization ability was evaluated by determining the amount of available soluble phosphate in culture using phosphomolybdate method [13]. After periode incubation, 2 mL of suspension were collected and centrifuged at 10000 rpm for 10 minutes. Then, 1 mL of the supernatant was added with 0.1 mL of chlorostannous acid and 10 mL of chloromolybdate reagent. Then diluted using aquadest to 25 mL until homogenized, and incubated for ten minutes. The sample

absorbance was calculated using a UV-VIS spectrophotometer at 690 nm. A standard phosphate curve was used to measure solubilized phosphate concentration.

3. Result and Discussion

The results of the chemical parameters of tannery waste water are presented in Table 1. All parameters were above the threshold prescribed by the Indonesian government (IG) (2016). Chromium was detected around 5.76 ppm, which was higher than the amount prescribed by IG (0.6 mgL-1). Chromium is a major pollutant in the leather tanning industry [14], [15]. During the tanning process, the chromium used is not completely fixed by the skin and precipitate in wastewater [14]. The absorption of chromium is very low (50-70%) [16]

In this study, the chromium content was relatively low (5 mg), because the waste sample was taken from the final drainage effluent. This shows that even though the wastewater has been treated, the chromium content is still above the threshold. The value of BOD and COD was above the tolerance limit of 50 and 110 mgL-1 respectively. The high BOD, COD, and COD/BOD ratio indicates that the tannery waste water contains low biodegradability of pollutants and high pollutant level. The main components in the waste are the products of protein and fat degradation [1]. The waste has a high pH (8.52) due to the using of alkali during the beam house process. The hides are treated with an intensely alkaline solution of lime (Ca (OH) 2) and sodium sulfide (Na2S) to ensure the removal of hair and wool (depilation process). Later, the skins are swollen in the liming process by immersing them in a strong bleach. [1]. Although the chromium in the waste is relatively low, the level in the soil is very high at 1333 ppm (Table 2). This happen because the amount of waste often exceeds the capacity of the reservoir so that it overflows into the surrounding land. High exposure to chromium in soil has a negative impact on plants, because it is toxic. Chromium resulted in decreased germination percentage, reduced root length and dry weight, and plant height [17]. To reduce the occurrence of soil contamination, waste water management must be carried out properly.

Table 1: Chemical Characteristics of the tannery waste water.

No	Characteristic		Value
1	Total Cr	(mg L^{-1})	5.7595
3	COD	(ml L^{-1})	2225.6
4	BOD	(ml L^{-1})	793.72
5	pH		8.52

Table 2: Chemical Characteristics of the soil surrounding the tannery.

No	Characteristics		Value
1	Chrome total (Cr)	(mg L^{-1})	1333
2	Carbon organic	(%)	4.4882
3	CEC	(cmol $^{+}$ kg^{-1})	25,7

3.1. Assay of viability of soil Phosphate Solubilizing Bacteria (PSB) community exposed to the waste at concentrations of 0, 30, 60, and 100%.

Environmental factors such as the availability and type of carbon, energy sources, the presence of heavy metals, toxic compounds and extreme pH affect the viability of microorganisms in their environment. Bacteria grow quickly in favorable environmental conditions resulting in high cell density and in unfavorable environmental conditions cell growth is inhibited so that the number of cells decreases. Exposure to waste has a negative effect on the viability of the soil PSB community (Figure ?? and 3). The density of PSB in polluted and unpolluted soil is very low, around 10^2 cfu/gr. In polluted soil, although the total Cr content was high, the amount of PSB in polluted soil was not different from that in unpolluted soil. This is because the soil organic matter content is high, so the toxicity of Chromium is reduced, it is adsorbed on the organic matter.

Exposure to waste starting at a concentration of 30% into the soil has shown a decrease in cell viability. The growth rate is negative. A negative growth rate indicates that the number of bacteria that grows is less than the number of bacteria that die. This is due to very slow cell growth due to environmental factors that do not support cell activity. The decrease in growth rate occurred because the added waste had chromium content above the threshold, high pH, and high COD/BOD ratio.

Chromium can be a pollutant in the environment in two forms of oxidation states, but the stable form in environment only Chromium (III) and Chromium (VI). Chromium (III) is more insoluble and immobile, while the hexavalent form is more toxic to life organisms. Chromium (VI) have high solubility and more mobile than Chromium (III) [18]. Although the amount of Chromium (III) that can enter the cell is very limited, after entering the cell, intracellular cationic Chromium (III) interact with phosphate in DNA and blocked DNA replication, and lead to missed RNA transcription. Chromium (III) can interact with some reducing compounds producing unstable intermediates as well as free radicals [18], [19].

The waste has high BOD and COD, indicating that the organic matter content of the waste is high. However, a high COD/BOD ratio illustrates that the types of organic matter

contained in the waste are difficult to degrade, so they cannot be used as a source of nutrients that support the growth of microorganisms, and may even be toxic. High pH (8.52) also inhibits bacterial growth.

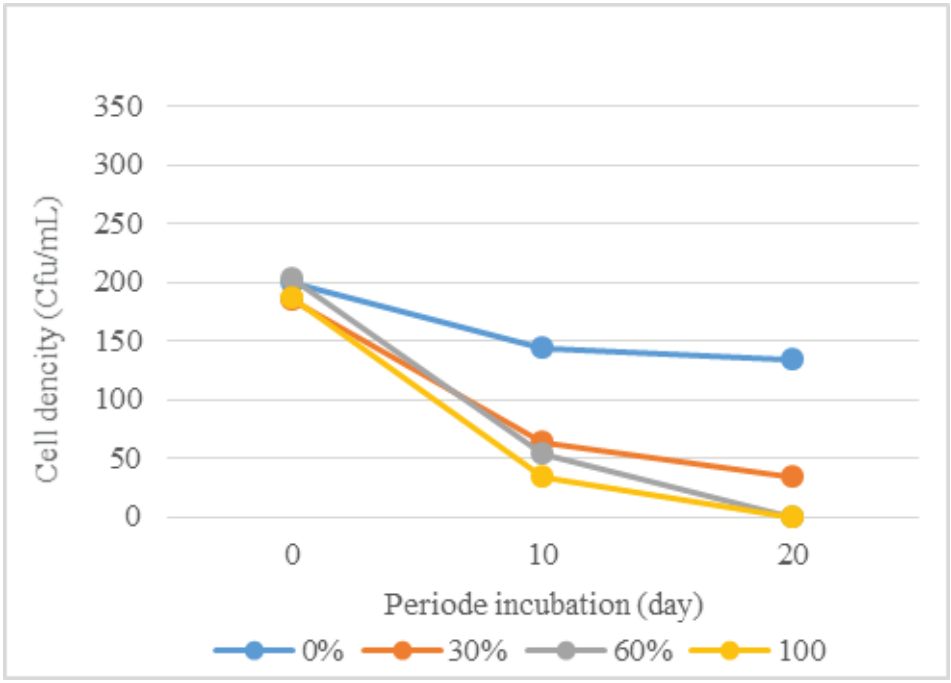

Figure 2: Tue Growth of unpolluted soil PSB community supplemented with tannery waste at concentrations of 0, 30, 60, or 100%.

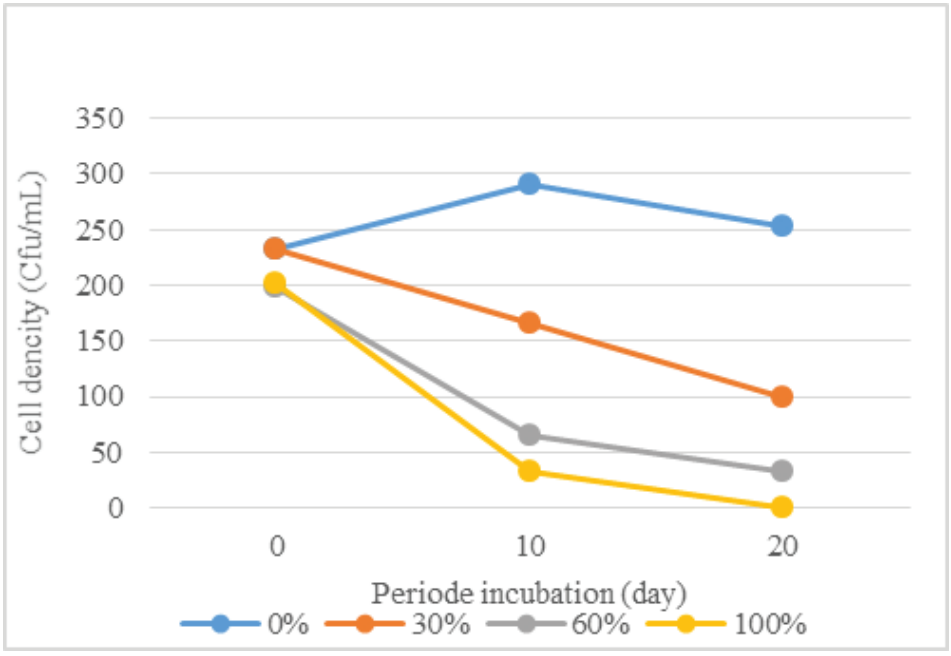

Figure 3: The Growth of polluted soil PSB community supplemented with tannery waste at concentrations of 0, 30, 60, or 100%.

3.2. Isolation and selection of PSB

In the present study, the soil was plated in PKV agar plate for PSB. Based on the diversity in the solubilization index, among the 7 isolates obtained, two isolates were selected for further analysis, which had the lowest solubilization index (RP-1 isolate) and the highest solubilization index (RP-2 isolate) (Table 3). The diameter of the RP-1 isolate colony was wider than that of RP2, indicating that the RP-1 isolate had a higher growth rate than the RP-2 isolate. However, based on the solubilization index value, the P solubilization ability of isolate RP1 was lower than that of isolate RP-2. Based on the differences in the characters of the two different isolates, further tests were carried out on the ability to grow and dissolve phosphate in Pikovskaya's broth which was added with waste until the final concentrations reached 0, 30, 60, and 100%.

Table 3: Colony morphology and Solubilization index of RP-1 and RP-2 isolates.

Characteristics	(A)RP-1 isolate	(B) RP-2 isolate
Form	Circulair	Amoeboid
Color	cream	Cream
Elevation	Convex	Convex papillate
Margin	Entire	Lobate
Structure	Smooth	Coarsely granular
Diameter of colony (mm)	7,1	4,0
Diameter of halo zone (mm)	10,0	12,0
Solubilization index (SI)	1,4	3,0

3.3. Assay of viability and P solubilizing activity of isolates

Cell viability was determined based on the growth of each isolate in Pikovskaya's broth which was added with waste. P solubilizing activity was determined based on the amount of dissolved P in water. The results revealed that waste exposure had a negative impact on development (Figures 4 and 5) and phosphate solubilization ability of the two isolates (Figures 6). Isolate RP-1 which has a higher growth rate than RP-2 has better viability than isolate RP-2. At concentrations up to 100% it is still viable, although its cell viability decreases with increasing levels of waste. This indicates that isolate RP-1 is more tolerant of exposure to waste than isolate RP-2. When associated with the levels of Chromium in the waste, the isolate RP-1 was more resistant to Chromium than isolate RP-2. Some bacteria that are tolerant to Chromium (VI) can accumulate or reduce to Chromium (III). The bacteria can have certain mechanisms so that they are resistant, these mechanisms include reducing absorption of Chromium (VI) entering through the

cell membrane reducing biosorption. In most cases the mechanism of resistance to metals controlled by genes contained in plasmids [1].

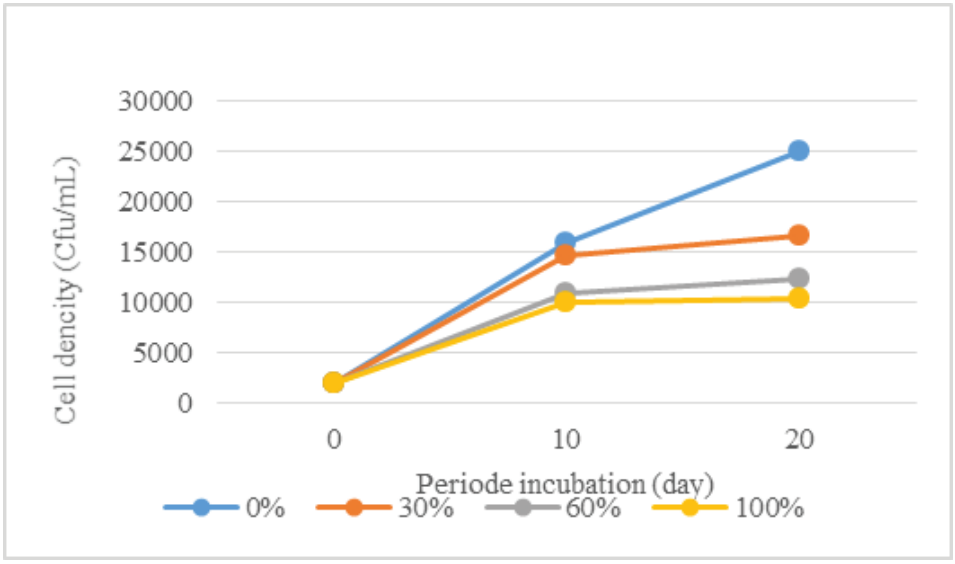

Figure 4: Growth of RP-1 isolate in Picovskaya's broth medium supplemented with tanne1y waste at concentrations of 0, 30, 60, or 100%.

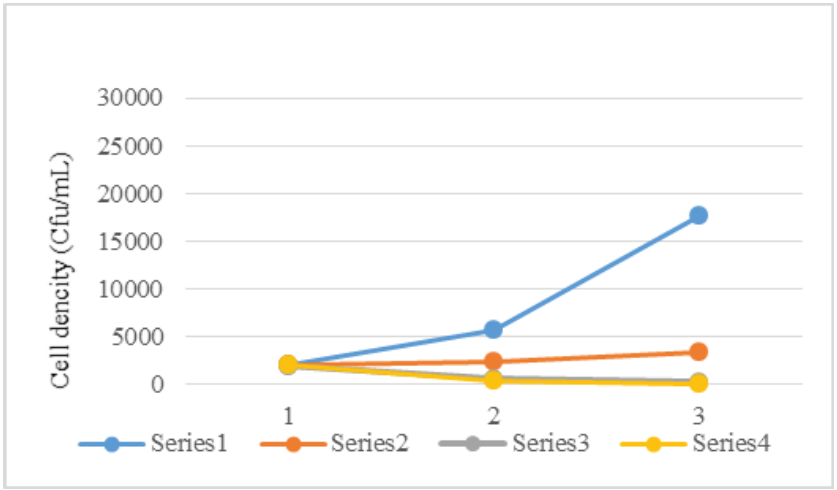

Figure 5: Growth of RP-2 isolate in Picovskaya's broth medium supplemented with tanne1ywaste at concentrations of 0, 30, 60, or 100%.

Figure ??. P-solubilization activity of the RP-1 and RP-2 isolates on Pikovskaya's broth medium supplemented with tannery waste at concentrations of 0, 30, 60, and 100%,

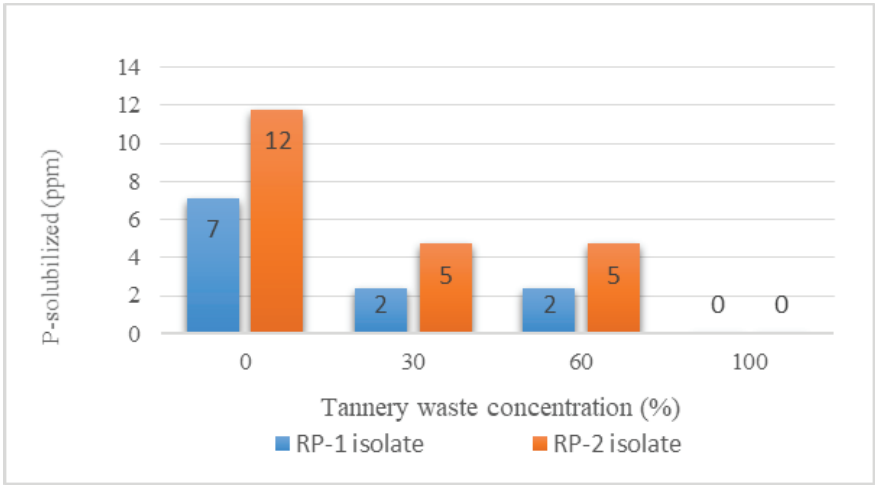

Figure 6: P-solubilization activity of the RP-1 and RP-2 isolates on Pikovskaya's broth medium supplemented with tannery waste at concentrations of 0, 30, 60, and 100%.

4. Conclusion

The experiment showed that exposure to effluents decreased the viability of PSB in the soil. Exposure to waste also negatively affected cell viability and the phosphate solubilizing ability of isolates. The application of PSB on soil exposed to tannery waste needs to be considered if the pH and COD/BOD ratio are high and the Chromium level is above the threshold because this results in a decrease in the ability of bacteria to dissolve phosphate.

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