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Phytoremediation of Lead Contaminated Soil Using Sorghum Plant in Association with Indigenous Microbes

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ABSTRACT

Heavy metals are discharged in large quantities in both land and water bodies, causing long-term damage to living organisms. Phytoremediation is an effective way to address this problem. The goal of this study was to identify how lead resistant microorganisms affect the growth of sorghum plant, both in the presence and absence of lead. For this purpose, lead resistant microbes were isolated to investigate the growth and concentration of lead in the sorghum plant. Isolated species were inoculated with lead containing media in different concentrations, such as 300, 400, 500, and 600 µg/ml concentrations. Highly lead resistant bacterial isolates were selected and inoculated with sorghum seeds under typical environmental conditions in small pots, with and without lead contamination (300 mg/Kg). In the presence of lead resistant bacteria, efficient growth was observed with less concentration of lead in the plants. Promising results were observed in the presence of GS3 and IS2 isolates. The current study showed that lead tolerant bacterial isolates are very helpful to degrade lead when grown with sorghum seeds. Furthermore, it also enhances the growth of sorghum plant.

Keywords: bioremediation, heavy metals, lead, phytoremediation, sorghum

INTRODUCTION

Soil is a mixture of both organic and inorganic matter which supports plant growth. It is an important natural resource necessary for the survival of human beings, as it contains minerals, organic matter, water, and energy [1]. It serves as a gene pool for a wide range of creatures. When lead stays in the environment for a long period of time, it affects soil and water adversely, since it cannot be removed or degraded easily from the environment. It has become a serious contaminant in urban areas due to its emission form automobiles. Phytoremediation is a technique used to remove or degrade heavy metals from the soil [2]. The current study revealed that phytoremediation is helpful in removing lead from the soil.

Sorghum is a major staple food grown in different parts of Pakistan. The current study was conducted to check the phytoremediation potential of sorghum with bacterial inoculation.

Heavy metal pollution occurs due to human activities, such as industrialization, use of fertilizer and pesticides, mining and metallurgical processes, and waste disposal. To remediate heavy metals from the contaminated soil, different types of phytoremediation techniques are used, such as phytoextraction. It is a cost-effective remediation technology in which plants are used to extract metals from soils and the extracted metals are translocated to the harvestable sections of plants [3]. Many studies found that certain varieties of high-biomass crops, such as Indian mustard (*Brassica juncea*), oat (*Avena sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), sunflower (*Helianthus annuus*), and ryegrass (*Lolium perenne*) are resistant to heavy metals [4]. Moreover, fast-growing willows (*Salix viminalis*) and poplars (*Populus sp.*) are good biomass producers and have a variety of features that make them potential candidates for phytoremediation [5]. One advantage of employing crops for phytoextraction is the potential for financial gain during the process. Sweet sorghum (*Sorghum bicolor*) is a tough C4 grass widely utilized as a pasture crop [6]. It is regarded as a promising plant because of its rapid growth and high biomass production.

2. MATERIALS AND METHOD

2.1 Sample Collection

Soil samples were collected from different sites of District Lahore, Punjab, Pakistan including industrial and agricultural area. All the collected samples were sieved (2 mm pore size) and their physiological characteristics (p^H and temperature) were determined [7].

2.2 Isolation of Lead Resistant Bacterial Isolates

Serial dilution of samples was followed by the isolation of strains by spread plate method on Luria agar plates. The plates were incubated at 37°C for 24 hours.

2.3 Screening and Selection of Isolates

Isolated strains of bacteria were inoculated in Luria agar plates having 200 µg/ml lead concentration. After incubation, visible colonies of bacterial isolates were further inoculated with different concentrations of lead containing media, that is, 300, 400, 500, and 600 µg/ml in test tubes and incubated for 48 hours. Bacterial isolates were selected for further experimentation. Different biochemical tests were performed to identify the selected bacterial isolates using IMViC, H_2S, Catalase, Indole, and nitrate reduction.

2.4 Pot Experimentation

2.4.1. Collection of soil samples for pot experimentation

For pot experiments, soil samples were collected from the agricultural area located in District Lahore, Punjab, Pakistan. Physiological characteristics, such as temperature, pH, and lead content were observed using atomic absorption spectrophotometer.

2.4.2. Collection and sterilization of sorghum seeds

Sorghum seeds were collected from the National Agriculture Research Centre, Islamabad, Pakistan. Surface sterilization of sorghum seeds was carried out by soaking them in 0.1% $HgCl_2$ solution for 10-15 minutes. After surface sterilization, the seeds were washed 2 to 3 times with sterilized water to remove any trace of mercuric chloride. Afterwards, the seeds were left soaked in the sterilized distilled water for 30-40 minutes [8].

2.4.3. Seed inoculation with bacterial isolates

About 40 ml of 15-day old bacterial isolates were centrifuged at 16000 rpm for 10 minutes. Then, the pellets were suspended in 24 ml autoclaved saline water. Afterwards, sterilized sorghum seeds were soaked in bacterial isolate suspensions for 25-30 minutes [9]. The seeds were soaked in autoclaved distilled water for some duration (for control) to check the effect of lead resistant bacterial isolates on the growth of the plants. Inoculated seeds were sown both in the soil containing 300 mg/kg lead and in the soil without lead (acting as control), with three replicas each.

2.4.4. Measurement of sorghum plant parameters after harvesting

Harvesting was done after 15-20 days. Different growth parameters and lead concentrations of sorghum plants were also measured. Growth parameters included root and shoot fresh weight, root and shoot length, number of roots and leaves, and the weight of aggregates attached to the roots. All of these were measured and analyzed.

2.4.5. Concentration of lead in sorghum plant

Lead absorbance of sorghum plant was determined by atomic absorption spectrometer. Afterwards, the concentration of lead within the plant was calculated using the following formula [10]:

Concentration of lead = absorbance of lead × total volume of sample / dry weight of sample.

3. RESULTS AND DISCUSSION

3.1 Characteristics of Soil Sample

Physiological characteristics of the collected soil samples were determined. S1and S4 were observed to be 9, whereas S2 was observed to be 8.5 and S3 was 8. The temperature of samples S1, S3, and S4 was observed to be 20°C and of S2 it was 25°C.

3.2 Selected Bacterial Isolate

The selected bacteria were grown on different concentrations of lead containing media, as indicated in **Table 2**. Bacterial isolates GS3, RS2, and IS3 were *bacillus*, while IS2 and AS1 were *cocci*. Names were given to them after their morphological (micro and macroscopic) characterization. Biochemical tests including MR, VP, SIM, Motility, H_2S, Catalase, Indole and nitrate reduction were performed for the identification of selected bacterial isolates, as described in **Table 1**.

Table 1: Biochemical Characterization of Isolates on Lead Containing Media

Isolates ID	MR	VP	Indole	Catalase	Nitrate reduction	SIM	H_2S	Morphological characteristics of microorganism
GS3	-	+	-	+	+	+	-	Bacilli
RS2	-	+	-	+	+	+	-	Bacilli
IS2	+	+	-	+	+	-	-	Cocci
IS3	-	+	-	+	+	+	-	Bacilli
AS1	+	+	-	+	+	-	-	Cocci

3.3 Physiological Characteristics of Soil Samples Collected for Pot Experimentation

Physiological characteristics of soil samples used in pot experimentation are indicated in **Table 6**. It explicates that the pH of soil was 8, the temperature of soil was 26°C, the natural concentration of lead in the soil was 103 mg/kg, and by adding lead to the soil the concentration became 349 mg/kg. Soil pH, among other properties of the soil, has a strong impact on the speciation and solubility of metal, both in soil solution and in the soil itself [11]. Lead is an immobile metal in the soil [12] because it readily forms a precipitate of low water solubility within the soil matrix. Moreover, it is not readily bioavailable in many instances. As a result, depending on soil properties, such factors can function separately or in combination, thus altering the behavior of lead present in the soil as well as the rate of its uptake by plants.

3.4 Effects of Bacterial Isolates on Different Sorghum Plant Parameters

Inoculated sorghum seeds were sown to check the effect of bacterial isolates and to check plant growth in the presence and absence of lead contaminated soil. Improvement in growth parameters (root shoot length, fresh weight of root shoot, number of leaves and roots, and weight of aggregates attached to the roots) was observed for inoculated seeds with lead tolerant bacterial isolates. Moreover, growth enhancement was possibly due to the release of phytohormones. Growth produces siderophores and also solubilizes and enhances the nutrient uptake that are released due to rhizobacterial isolates [13]. The difference in the efficacy of the isolates was possibly due to their different potential to colonize roots [14].

3.5 Shoot and Root Fresh Weight (g)

Table 1 shows that reduction in shoot fresh weight occurred due to lead contamination, as compared to plants grown with lead tolerant bacteria. Moreover, it shows that shoot weight increased by 45% when grown with GS3 and RS2 bacterial isolates. Lead resistant bacterial isolates increase the weight of plants, whereas the absence of lead increases the shoot weight up to 45.75%. Furthermore, IS3 and AS1 decreased up to 66% and 29% of shoot weight respectively as compared to control (plants grown without the inoculation of bacterial isolates). However, shoot weight increased when the plant was grown with bacterial isolates in the absence of lead, that is, GS3 increased the weight of shoot up to 11%. Whereas, IS2 and IS3 decreased its weight by 14% and 17% respectively as compared to control, (plants grown without lead and bacterial isolates). Arguably, the application of lead tolerant bacterial isolates improves the shoot fresh weight. Moreover, bacterial isolate (GS3) increases the shoot weight more than other bacterial isolates. Lead tolerant bacteria improves their growth and it could be due to rhizobacteria that releases phytohormones. Variation in bacterial isolates may be due to their different colonization ability in roots or different natural potential [15].

Table 1: Statistical Analysis of Sorghum Plant Root and Shoot Fresh Weight with Bacterial Isolates

Treatment	**Shoot Fresh weight (g)**		**Root Fresh weight (g)**	
	With lead	**Without lead**	**With lead**	**Without lead**
Control	0.24±0.04(a)	1.15±0.06 (bc)	0.31±0.04 (abc)	0.61±0.03 (d)
GS3	0.35±0.01 (a)	1.28±0.04(bc)	0.19±0.04(abc)	0.62±0.04(d)
RS2	0.35±0.01(a)	1±0.054(bc)	0.11±0.03 (ab)	0.15±0.05(ab)
IS2	0.23±0.05 (a)	0.98±0.05(bc)	0.17±0.03(abc)	0.09±0.013 (a)
IS3	0.08±0.011(a)	0.95±0.06(b)	0.35±0.02 (abcd)	0.28±0.03(abc)
AS1	0.17±0.07(a)	1.2±0.035(bc)	0.14±0.04 (ab)	0.17±0.03(abc)

Root fresh weight was also affected by lead contamination, although a very minor change was observed. Bacterial isolates were not very effective in enhancing root weight. Only about 12% increment was observed with IS3 bacterial isolate. Whereas, all other bacterial isolates showed a decrease (up to 64%) of root fresh weight. However, root fresh weight increased when exposed to GS3 bacterial isolate (up to 2%) in the absence of lead. Whereas, all other bacterial isolates did not affect root weight which decreased up to 72% as compared to control, that is, without lead and bacterial inoculation. The results clearly show that bacterial isolates affect the root weight negatively. Improvement in root fresh weight was observed (up to 12%) when exposed to lead tolerant bacteria, that is, IS3 in soil contaminated with lead as compared to control (without the inoculation of bacteria).

3.6 Shoot and Root Length (cm)

Inoculation with lead tolerant bacteria showed their growth promoting potential and increased the shoot length, with and without lead contamination, as compared to their respective controls. The highest percentage increment was 21% when exposed to AS1 bacterial isolate, while the lowest percentage increase was 5% with GS3 bacterial isolate. Whereas, the rest of the bacterial isolates increased the root length between the highest and the lowest percentage points as compared to control, that is, in the presence of lead but without bacterial isolates. These results showed that bacterial isolates enhance the shoot length. However, even without lead contamination, bacterial isolates enhance plant growth. It indicates that bacterial isolates help the sorghum plant to grow and enhance plant length. Moreover, 28% of increment was observed with GS3 bacterial isolate, while IS2 showed 4% increment in shoot length. A small amount of lead is naturally present in the soil. Due to its presence, these lead tolerant bacterial isolates affect shoot length and improve shoot growth.

Table 2: Statistical Analysis of Sorghum Plant Root and Shoot Length with Bacterial Isolates

Treatment	Shoot length		Root length	
	With lead	**Without lead**	**With lead**	**Without lead**
Control	3.38±0.25(hi)	4.55±0.21(i)	1.3±0.1(cdefg)	1.5±0.12(abcd)
GS3	3.55±0.12(efgh)	5.85±0.16(j)	1.8±0.11(abcde)	1.8±0.17(abcd)
RS2	3.75±0.12(fghi)	4.9±0.26(ghi)	2.43±0.15(hi)	1.5±0.11(bcdefg)
IS2	3.7±0.11(ab)	4.73±0.04(fghi)	0.65±0.07(a)	2.85±0.16(i)
IS3	3.8±0.22(a)	4.93±0.22(ghi)	1.6±0.13(defg)	2.28±0.09(hi)
AS1	4.1±0.22 (b)	5.58±0.22(i)	1.5±0.15(defg)	1.6±0.05(defg)

Root length increased when plants were grown with bacterial strains but in the presence of lead; however, only bacterial isolate IS2 showed a decrease in root length (up to 50%). The percentage of increment was 86% with the bacterial isolate RS2, while GS3, IS3, and AS1 showed an increment of 38%, 23%, and 15%, respectively as compared to control, that is, without any bacterial strain. Lead tolerant bacterial isolates increased the root length even in the absence of lead contamination. In this regard, the highest percentage increment was 90%. It was observed with IS2 bacterial isolate. Whereas, the lowest percentage increment was observed at 7% with AS1 bacterial strain. All other bacterial isolates increased the root length between the highest and the lowest percentage increment as compared to control, that is, without lead contamination and bacterial isolates. The data in **Table 2** shows that bacterial isolates enhance root growth.

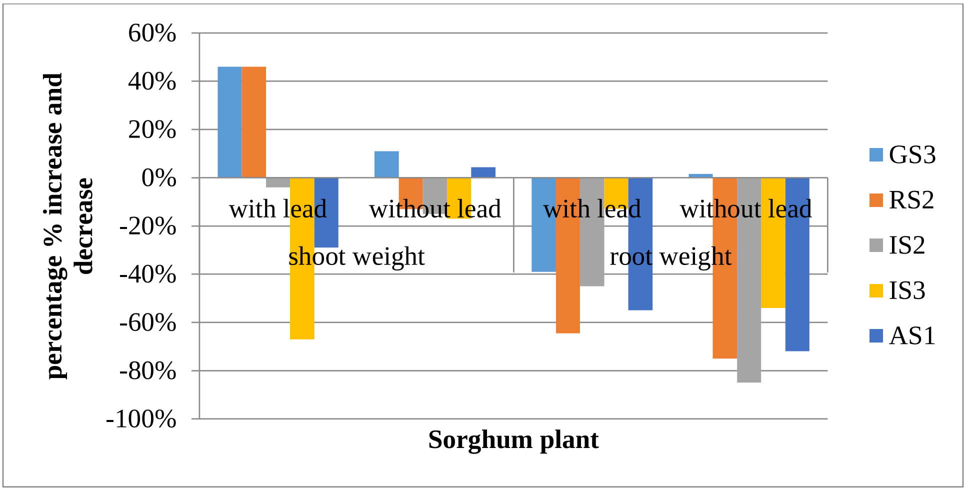

Figure 1. Fresh weight of the roots and shoots of sorghum plant (in percentages)

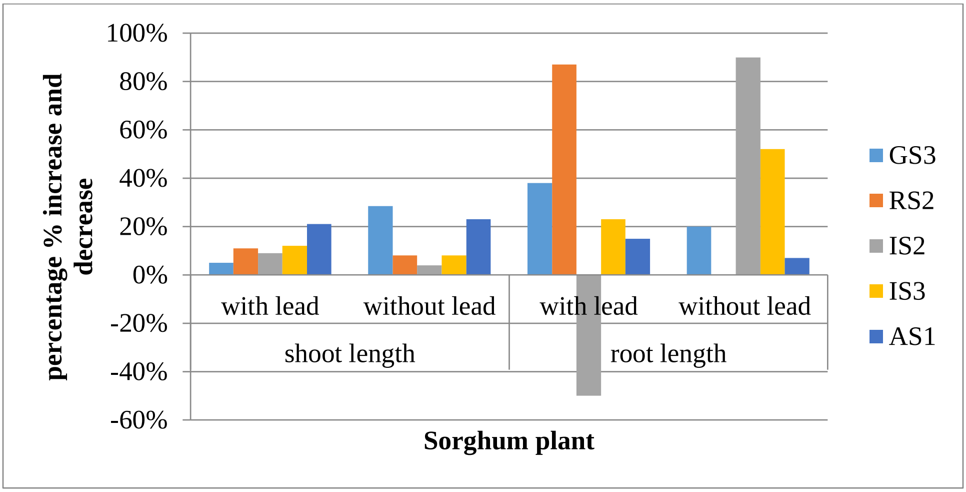

Figure 2. Sorghum plant root and shoot length (in percentages)

3.7 Number of Roots and Leaves

Lead could be present in the soil for a long period of time. However, soil bacteria may develop resistance to its toxicity. In the current study, it was found that the number of leaves and roots varied with bacterial isolates. The number of roots per plant increased in lead stress by the application of lead tolerant bacterial isolates. An increment of about 83% in the root numbers of roots was observed in the presence of lead with bacterial isolate GS3. All bacterial isolates improved the percentage of roots from 33% to 83%. Lead tolerant bacteria promoted root growth; however, in the absence of lead 28% increase in the numbers of roots was observed with bacterial isolate IS3. Whereas, AS1 showed a slight decrease at 7% as compared to control, that is, without bacterial isolates and in the absence of lead. This shows that bacterial isolates increase numbers of roots in the presence and absence of lead. **Table 3** shows the positive response of lead tolerant bacterial isolates.

Table 3: Statistical Analysis of the Number of Sorghum Plant Roots and Leaves with Bacterial Isolates

Treatment	No of roots		No of leaves	
	With lead	**Without lead**	**With lead**	**Without lead**
Control	3±0.11(ab)	3.5±0.25(abc)	2.5±0.15(a)	2±0.15(a)
GS3	5.5±0.15 (c)	3.5±0.15(abc)	2±0.12(a)	3.5±0.23(ab)
RS2	4±0.21 (abc)	5±0.11 (bc)	2±0.25(a)	2.5±0.09(a)
IS2	4.25±0.35(abc)	3.5±0.25(abc)	2.5±0.31(a)	3±0.02(b)
IS3	4.25±0.25(abc)	4.5±0.15(abc)	2.5±0.02 (a)	3.5±0.2(b)
AS1	3.5±0.35(abc)	3.25±0.13 (ab)	3±0.048(abc)	2±0.06(abc)

On the contrary, **Table 3** shows little effect regarding the number of leaves. An equal growth with bacterial isolates IS2 and IS3 was observed, while 20% of increment was observed with AS1 bacterial isolate. Moreover, GS3 and RS2 showed 20% percentage point decrease as compared to control, that is, without bacterial isolates. However, all bacterial isolates showed an increase in the number of leaves when the plant was grown without inoculated lead contamination. The highest percentage increment was 75% as compared to control, that is, without lead contamination. Hence, it was concluded that bacterial isolates greatly affect the number of leaves and enhance plant growth. This study showed that bacterial isolates affect the growth of leaves and roots.

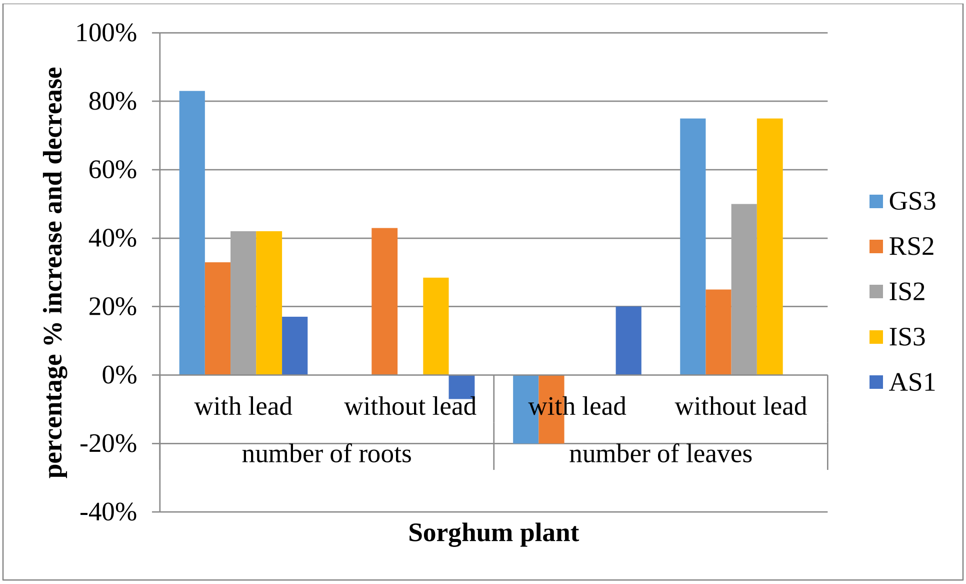

Figure 3. Number of roots and shoots of sorghum plant (in percentages)

3.8 Concentration of Lead in Sorghum Plant

Table 4 shows a significant effect of inoculation on lead concentration in sorghum plant. It was observed that the inoculation of lead tolerant bacteria reduced lead concentration in the plants (grown at the given level of lead) as compared to the plants grown in non-inoculated lead contaminated soil. The concentration of lead decreased with the presence of bacterial isolates as compared to control, that is, without bacterial isolates. Bacterial isolates adversely affected the concentration of lead in sorghum plant. This is due to the fact that bacterial isolates reduced the translocation of lead within the plant [15]. Bacterial isolate IS2 showed more concentration in the plant than other bacterial isolates. About 42% of the highest level of lead concentration was observed with IS2 bacterial isolate Whereas, IS3 and AS1 showed the lowest concentration of lead, that is, 72% and 66%, respectively in the plants as compared to control, that is, with lead and without bacterial isolates. However, all bacterial isolates decreased lead concentration in the plants without inoculated lead contamination, except IS3 bacterial isolate which showed an increment in the lead concentration of the plant (up to

7.3%). The reduction in lead concentration showed that lead tolerant bacterial isolates were able to reduce the concentration of lead in the plants as compared to control (without bacterial inoculation). **Table 4** shows that bacterial isolates affect lead concentration in sorghum plant positively.

Table 4: Statistical Analysis of the Concentration of Lead in Sorghum Plant with Bacterial Isolates

Treatment	Concentration of lead	
	With lead	Without lead
Control	238.5±0.65(c)	41.3±0.30(a)
GS3	97.10±2.11 (d)	27.7±0.46 (e)
RS2	92.1±2.09 (e)	31±1.40(c)
IS2	138.1±1.49(e)	38.5±0.41(b)
IS3	65.6±1.29(b)	44.47±0.78 (d)
AS1	80.5±0.78 (a)	34.7±1.23 (a)

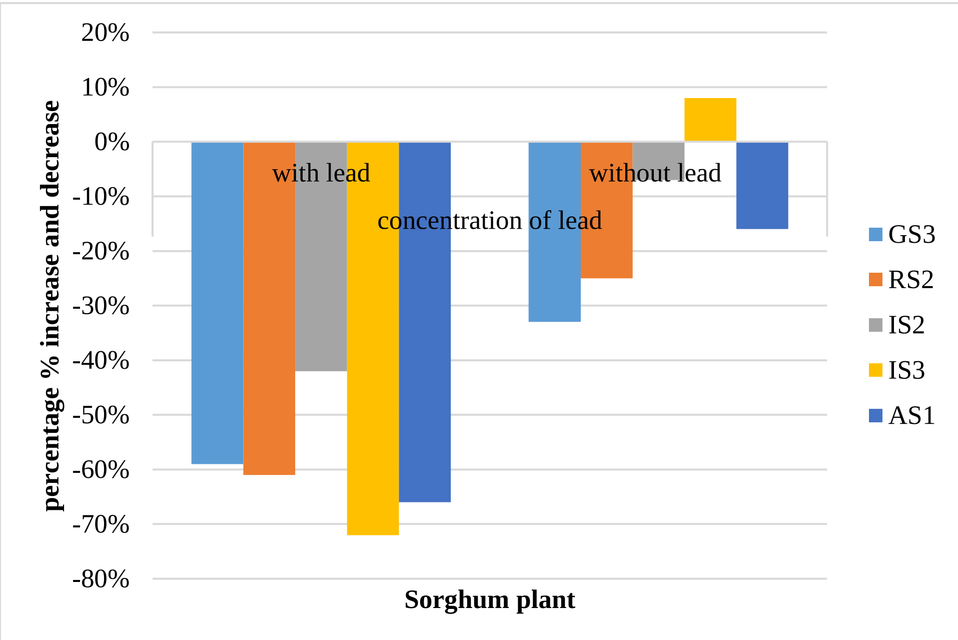

Figure 5. Concentration of lead in sorghum plant (in percentages)

4. CONCLUSION

The study revealed that lead resistant bacteria showed promising results in eliminating lead contamination from the soil along with a synergistic effect in the growth of sorghum. This study opens a window towards the use of plants with bacterial species that may increase their development and growth and also play a key role in bioremediation.

4.1 Future Prospects

Phytoremediation is a cost-effective method to remove heavy metals from the soil. This technique can be used in the future to remediate heavy metals near industrial areas where crops are grown. The crops that contain heavy metals also affect our health adversely and cause serious disorders. However, if bacterial inoculations are used with the seeds to grow crops, then it helps to reduce metal contamination from the soil and such crops can be used without causing serious health issues. On the other hand, there are fields near roads. The crops grown in these fields also contain heavy metals. This remediation technique can be employed to reduce the metal contaminant in those crops as well. Indeed, phytoremediation is an innovative technology which is very useful and could be used in the future.

Conflict of Interest Statement

There is no conflict of interest among author of this article.

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