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Investigating the Effects of Salicylic Acid and Rice Husk Ash on Pb and Cd Concentration in Pinto Bean Plants Grown in Soil Contaminated with Diesel Oil and Heavy Metals

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Background: This study was conducted to investigate the effects of salicylic acid and rice husk ash on Pb and Cd concentration in pinto bean plants grown in soil that was contaminated with both diesel oil and heavy metals.
Methods: The treatments in this study included the application of rice husk ash to soil at concentrations of 0 %, 4 %, and 8 % (W/W) as well as the foliar application of salicylic acid to pinto bean plants grown in soil contaminated with diesel oil, Cd, and Pb at concentrations of 0 %, 4 % and 8 % (W/W). After a growth period of 90 days, the pinto bean plant was harvested and the concentration of Pb and Cd in the plants was measured using Atomic absorption spectroscopy. In addition, the extent of diesel oil bio-degradation in the soil was determined.
Results: The results indicate a significant reduction in the concentration of Cd and Pb in pinto bean plants (by 11.7 and 15.4 %, respectively) due to the application of rice husk ash (8 % (W/W)) in the soil. Furthermore, the bio-degradation of diesel oil in the soil was found to be significantly enhanced, while the activity of plant enzymes APX and POX was observed to be decreased.
Conclusion: The interaction between rice husk ash and salicylic acid the reduction in Pb and Cd concentration was significantly influenced by, while the bio-degradation of diesel oil in the soil increased.

1. Introduction

Numerous industries generate a substantial volume of waste daily, which poses a significant challenge in terms of disposal. Of particular concern are waste materials such as rice husk ash, wood ash, and plastic waste, which contribute to the crisis of waste destruction [1, 2]. Nowadays, waste management has become increasingly critical. In our country, on average, 30 % of agricultural products are wasted due to inadequacies in the storage, transformation, and distribution system, even though 70 % of the country's agricultural waste is organic and vital, and has the potential to be repurposed for soil generation [3]. Consequently, despite the significant potential of the agricultural sector, the main challenge facing the agricultural structure is the lack of proper organizational management and a lack of motivation for optimal and sustainable resource utilization [4]. The use of ashes has been a prevalent practice in the cement and concrete industries for several decades. However, in recent years, research has demonstrated the efficiency of these ashes in mitigating the mobility of trace elements and pesticides in soils [5]. Due to their alkaline and acidic properties, ashes can serve as soil amendments that improve physicochemical properties. Specifically, they enhance the

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cation exchange capacity, water retention capacity, and nutrient availability of soils, thereby promoting plant growth. However, the effectiveness of these compounds is contingent upon the state of soil physicochemical properties [6]. In research conducted on the use of wood ash for agriculture and forestry purposes, it has been shown that wood ash can function as a liming agent and facilitate nutrient cycling [7]. Wood ash can be used in forested regions to restore nutrient balance nutrients and offset deficiencies caused by leaching [8]. Using wood ash can increase soil acidity and decrease the solubility of heavy metals by pH level modulation [9]. On the other hand, recent research at the Korea Institute of Technology Research has focused on the potential of rice paddy husks, which contain approximately 50 % carbon materials and over 30 % silica. In addition to its use as compost, waterproofing material, and solid fuel, rice paddy husks can also be employed as a raw material for the production of activated carbon. The application of activated carbon in soil has been found to enhance soil sorption capacity, thereby facilitating the absorption of heavy metals [10]. Adsorption is a transfer process of an element from the liquid phase to the solid phase surface. This process is governed by the chemical interactions between the element and the surface of the absorber [11]. A study was conducted to evaluate the absorption capacities of heavy metals by various absorbents, including corn wood, paddy shell, peanut shell, human hair, wheat bran, and sugarcane bagasse. The results indicated that corn wood exhibited the highest heavy metal sorption capacity for heavy metals [12]. The accumulation of heavy agricultural products due to pollution is one of the environmental issues that threaten the life of plants, animals, and humans. These heavy metals can accumulate over time and impact the food chain by reducing plant growth, crop yield, and ultimately, human health [13]. Abdulrahimi and Ghorbanzadeh conducted a study on the efficacy of bentonite, as well as a natural and modified rice paddy, in immobilizing cadmium (Cd) and its impacts on various biological characteristics of the soil. Their findings revealed that the use of sorbent material can serve as an effective management strategy for immobilizing Cd and improving the overall biological condition of soil [14]. The concentration of heavy metals in soil strongly influences their uptake by plants. Due to the excessive use of phosphate fertilizers containing Cd in recent years, a decline in the quality of various agricultural products such as vegetables, wheat, potatoes onions, and others has been observed [15]. Cd is eliminated from the human body′s digestive system in minimal amounts, and its biological half-life is reported to range from 10-30 years [16, 17]. Elevated Cd concentration in the human body can initially cause damage to the liver and pancreas. Symptoms of lead (Pb) poisoning may include increased urination, a subtle increase in blood Pb levels, gastrointestinal complications, weakness and fatigue, neurological complications, and finally, death [18]. Nowadays, the accumulation of Cd in lands and agricultural products has emerged as a major environmental issue in our country. The entry of heavy metals into the food chain of plants can cause severe damage and poses a serious threat to human health [19]. Two primary mechanisms by which heavy metals can exert their toxicity in plants include the production of reactive oxygen species (ROS) through oxidation reactions, and the obstruction of essential functional groups of biomolecules, thereby impeding antioxidant activities [20]. Therefore, it is crucial to use effective methods that can mitigate the negative effects of heavy metal toxicity and prevent their absorption by the plant. In this regard, several methods have been proposed to improve seed germination and reduce the negative effects of environmental stress, one of which is seed pretreatment [21, 22]. Seed pretreatment can significantly enhance the quality of seed production. Primed seeds exhibit faster germination and complete their different biological stages more rapidly than untreated seeds [23]. This process can reduce the damage caused to the primed seeds during germination and ultimately result in healthier plants that are better able to withstand adverse environmental conditions. Moreover, seed priming has been shown to significantly increase seed germination and growth rates under unfavorable environmental conditions. The primary objective of seed pretreatment is typically to augment the rate and percentage of seed germination and emergence and to improve the establishment of seedlings even in adverse environmental conditions [24]. Given that heavy elements, such as Cd, are among the most significant environmental stresses encountered in modern agricultural ecosystems, researchers have prioritized finding ways to mitigate or prevent their harmful effects and reduce their absorption [25]. To date, various processes, including ion exchange, chemical precipitation, and reverse osmosis, have been proposed for heavy metal removal; however, their high cost is a significant limitation [26]. In industrial regions, there exists a concurrent problem of heavy metal pollution from petroleum compounds that poses a risk to human health, as well as the challenge of managing agricultural waste. Annually, significant amounts of soil mineral elements are depleted from agricultural fields due to crop production, with even greater amounts removed as plant residues. This massive release of plant material, which is a source of energy and food supply, results in the gradual deterioration of soil quality, particularly organic matter content in arid and semi-arid regions. The incorporation of plant residues back into the soil is a crucial component of agricultural ecosystems [27]. For this purpose, the use of compounds such as rice husk ash [28, 29] or salicylic acid [30], has been proposed as potential means of alleviating the detrimental effects of environmental stress and enhancing plant biomass and food chain health. Therefore, this study was conducted to investigate the efficacy of rice husk ash and salicylic acid in reducing the absorption of Pb and Cd by pinto bean plants grown in petroleum hydrocarbon-contaminated soil.

2. Materials and Methods

To investigate the impact of plant growth regulators and rice husk ash on the biodegradation of diesel oil in naturally

Cd (15 mg Cd/kg soil) and Pb (1200 mg Pb/kg soil) contaminated soil, a factorial experiment was conducted using a randomized complete block design. The experimental treatments, which were replicated three times, involved the application of rice husk ash at rates of 0 %, 2 %, 4 %, and 8 % (w/w) to the soil, as well as the foliar application of salicylic acid at concentrations of 0, 1, 1.5, and 3 mmol/L to plants grown in soil contaminated with diesel oil at rates of 0 %, 4 %, and 8 % (w/w). The diesel oil was applied to the soil at the aforementioned concentrations and allowed to equilibrate for one month. After this period, the soil was treated with rice husk ash at the aforementioned rates and placed into 5 kg plastic pots for further experimentation.

Table 1. Selected physicochemical characteristics of soil research

Characteristic	Unit	Amount
Soil texture	----	Loam
pH	----	7.3
EC	dS/m	0.6
$CaCO_3$	%	8
Total Pb	mg/kg	1200
Total Cd	mg/kg	15
Total Ni	mg/kg	ND
Cation exchange capacity (CEC)	meq/100 g soil	11.8

In this study, pinto bean plants (*Phaseolus vulgaris L. Cv. Khomein*) a common species in the study area that is known to be resistant to drought stress, were used. Seeds of pinto bean plants were obtained from the Agricultural Research Center's station in the province of Markazi, Iran. These seeds were first soaked in water, followed by 15-second immersion in 96 % alcohol and a one-minute soak in a sodium hypochlorite solution (1:10, v/v). The seeds were then sterilized with distilled water multiple times and germinated on quartz sand that had been moistened with distilled water. Salicylic acid foliar treatment was performed two weeks after plant germination [31, 32]. After a 90-day growth period, the concentration of Pb and Cd in the plants was measured using atomic absorption spectroscopy [33]. The availability of Pb and Cd in the soil was determined using the method described by Linsay *et al.* [34]. Diesel oil bio-degradation in the soil was assessed using the Besalatpour method [35]. The activity of the APX (Ascorbate Peroxidase) and POX (Peroxidase) enzymes were also measured by grinding leaf samples with a 10 cm^3 extraction solution (0.1 M phosphate buffer, pH = 7.5, containing 0.5 mM ethylenediaminetetraacetic acid and 1 mM ascorbic acid), and extracting the enzymes [36]. Soil microbial respiration was measured using the method described by Zamani *et al.* [37]. 15 g of soil samples were incubated in 500 mL glass containers with rubber stoppers for 7 days at 27 °C, with a test tube containing 10 mL of a 0.5 M NaOH solution inserted into the containers to capture the evolving CO_2. The trapped CO_2 was identified by titrating the excess alkali with HCl, and three glass containers without soil were incubated as controls [37].

2.1 Statistical analysis

The statistical analysis was conducted using SAS V. 9. 1. In order to assess differences between means, the least significant difference (LSD) test was applied. A significance level of 95 % ($P < 0.05$) was employed to determine whether any observed changes were statistically significant. To confirm adherence to the assumptions of the ANOVA model, normality was assessed using Shapiro-Wilcoxon's test normality, and equal variance was assessed using Levene's test. If necessary, data were transformed using $\log_{10}$ or square root functions of the analysis.

3. Results and Discussion

The soil sample did not receive any organic amendments and exhibited the lowest levels of Cd and Pb concentrations, as indicated in Table 2. Using The application of rice husk ash at 4 % and 8 % (W/W) significantly reduced the Cd concentration of the soil by 11.2 % and 15.6 %, respectively. Furthermore, the application of rice husk ash at the same rates led to a significant decrease in soil Pb concentration by 12.9 % and 14.1 %, respectively. This reduction t can be attributed to the role of rice husk ash in enhancing the sorption properties of the soil, thereby reducing the availability of heavy metals in the soil. The observed increase in cation exchange capacity (CEC) of the soil due to soil application of rice husk further supports these findings, as depicted in Figure 1.

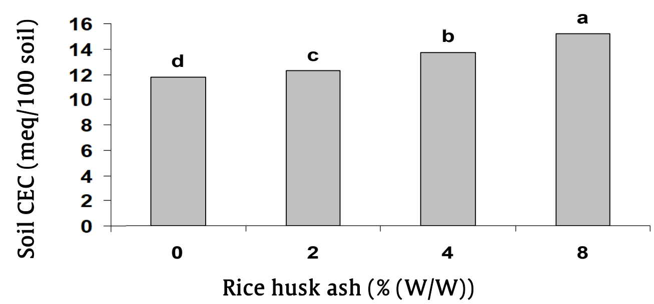

Figure 1. Effect of rice husk (% W/W) ash on soil CEC, different letters between columns show significant differences ($P < 0.05$)

Hamid and Tang (2019) evaluated the effectiveness of inorganic and organic treatments for immobilizing Cd and Pb in contaminated soil within a rice-wheat cropping system. The authors concluded that using these amendments had a significant effect on the immobilization of heavy metals in the soil as reported in their study [38]. These findings are consistent with the results obtained in our study. The application of salicylic acid via foliar spray significantly decreased the soil Pb and Cd concentrations. Specifically, using 1.5 mmol/l salicylic acid led to a significant decrease in soil Pb and Cd concentration by 12.7 % and 14.3 %, respectively. This reduction can be attributed to salicylic acid's role in promoting plant growth and thereby reducing the availability of heavy metals in the soil through immobilization. Salicylic acid, also known as orthohydroxybenzoic acid, is a phenolic derivative that is naturally present in many plant species. It is involved in regulating various physiological and biochemical processes, including plant signaling, thermogenesis or defense, and reaction to biotic and abiotic stress. Under heavy metal stress, salicylic acid interacts with several plant hormones, such as auxins, abscisic acid, and gibberellin [39]. In this regard, Jalali *et al.* (2021) investigated the effect of biochar and salicylic acid on soil heavy metal availability and concluded that using organic amendments had significant effects on the immobilization of heavy metals in the soil. However, the impact of other pollutants and their interactions on plant growth was not taken into account [40]. The combined application of rice husk ash and salicylic acid, both via soil and foliar spray, resulted in a positive effect on decreasing the soil heavy metal availability. Specifically, using 8 % (W/W) rice husk ash and 3 mmol/l salicylic acid significantly decreased soil Cd availability by 14.1 % and 18.3 %, respectively, while soil Pb availability was decreased by 17.2 % and 21.4 %, respectively. The presence of hydrocarbon pollution in soil had a significant impact on increasing the availability of Pb and Cd, as evidenced by the results of this study. Specifically, the soil sample with the highest level of diesel oil (8 % (W/W)) exhibited the greatest availability of Pb and Cd, which can be attributed to the impact of diesel oil pollution on soil microbial activity. The observed decrease in microbial respiration of the soil (as shown in Table 3) due to the increased level of diesel oil pollution further supports this finding. The concentration of Pb and Cd in the plant (Table 4) was impacted by both soil application of rice husk ash and foliar application of salicylic acid. Specifically, the application of 8 % (W/W) rice husk ash in soil significantly decreased Pb and Cd concentration of the plant by 13.1 % and 17.4 %, respectively. This reduction can be related to the soil sorption properties that are improved by organic amendments, which lower the concentration of Pb and Cd in plants. In this regard, Molaee and Shirani (2016) conducted a study on the effect of shrimp shells, vermicompost, and pistachio kernel on the Cd, Pb, and Zn uptake by corn in contaminated soil. They found that utilizing organic amendments significantly reduced the uptake of heavy metals by plants [41]. On the other hand, increasing soil pollution with diesel oil significantly increased the plant Pb and Cd concentrations. Our results showed that the concentration of Pb and Cd in the plant significantly increased by 13.2 % and 17.5 %, respectively with an increase in soil pollution caused by diesel oil from 0 to 8 % (W/W). This trend suggests that petroleum hydrocarbon can contribute to an increase in soil salinity, subsequently increasing the solubility of heavy metals in the soil. This is consistent with the findings of Abbaspour *et al.* (2008) who reported similar effects of petroleum hydrocarbons on soil salinity and heavy metal solubility [42]. It is generally recognized that salinity can increase the solubility and mobility of heavy metals in soil. This can occur through the formation of less charged or uncharged heavy metal complexes, as well as through increased competition between cations and heavy metals for adsorption on soil exchange sites [43]. Azadi and Raiesi (2021) investigated the effect of salinity on soil Cd availability and concluded that increasing soil salinity can increase heavy metal availability through competition between cations [44]. Our study found that the bio-degradation of diesel oil (Table 5) in the soil was significantly affected by the interaction of soil application of rice husk ash and foliar application of salicylic acid. Specifically, the use of 8 % (W/W) rice husk ash in combination with foliar application of salicylic acid (at a concentration of 3 mmol/L) on plants grown in Cd (15 mg Cd/kg soil) and Pb (1200 mg/kg soil)-polluted soil resulted in a significant increase in the bio-degradation of diesel oil by 16.2 %. This increase in diesel oil bio-degradation can be attributed to the ability of rice husk to improve soil sorption properties, such as soil CEC, which in turn can help reduce the toxicity of heavy metals and decrease their availability in the soil (as demonstrated in Table 1). The combined effect of rice husk ash and salicylic acid on the bio-degradation of diesel oil suggests that these amendments may have the potential for use in the remediation of hydrocarbon-contaminated soil. The significance of the foliar application of salicylic acid cannot be overlooked, as our results have shown that administering 3 mmol/l salicylic acid to plants cultivated in diesel oil-polluted soil (8 % (W/W)) has resulted in a significant 16.4 % increase in diesel oil bio-degradation. This effect is attributed to the salicylic acid application's facilitating role in augmenting the activity of soil microorganisms, as evidenced by the rise in soil microbial respiration (Table 2), and consequently heightening the bio-degradation of diesel oil in the soil. The application of rice husk ash to the soil and the administration of salicylic acid via foliar application has effectively reduced the activity of plant enzymes, namely Ascorbate peroxidase (APX) and peroxidase (POX). (Using 8 % (W/W) rice husk ash in diesel oil-polluted (4 % ((W/W)) has significantly diminished the APX and POX enzyme activity by 17.7 % and 21.9 %, respectively (Table 6). This outcome is likely attributed to the role of organic amendments in reducing Pb and Cd availability, consequently leading to a decrease in plant enzyme activity. In the case of foliar application of salicylic acid at a concentration of 3 mmol/l, the APX and POX enzyme activity have exhibited a decline of 18.2% and 24.3%, respectively. Soil contamination with heavy metals has the potential to significantly influence plant growth by changing physiological processes [45, 46]. The production of reactive oxygen species (ROS) is an indicator of plant damage, as ROS negatively affects cell membranes, nucleic acids, and chloroplast pigments. In response to induced heavy metals, plants produce antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and peroxidase (POD), as well as non-enzymatic substances such as cysteine (Cys), carotenoids, and ascorbate [47]. Previous research has shown that plants cultivated in highly contaminated soil with heavy metals have exhibited a 1.3-5.3-fold increase in CAT, APX, and GR antioxidant enzymes [48, 49], which is consistent with our findings.

Table 2. Effect of Rice husk ash and Salicylic acid on soil Pb and Cd availability (mg/kg soil)

Diesel oil (% (W/W)	Rice husk ash % (W/W): 0				2				4				8			
Salicylic acid (mmol/l)	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3
Soil Pb availability (mg/kg soil)																
0	120.2d*	115.4h	109.7n	105.4q	116.8g	113.3j	107.8p	102.7t	110.5m	109.2n	104.8r	97.3x	107.1p	104.8r	100.8v	93.6y
4	122.2b	117.2f	113.4j	108.8o	119.2e	115.8h	111.2l	107.8p	114.6i	110.7m	107.8p	102.6t	111.4l	109.3n	103.8s	99.5w
8	125.6a	121.6c	119.5e	112.4k	122.7	119.4e	115.6h	110.8m	119.2e	114.8i	111.4l	107.1p	114.7i	112.2k	107.4p	102.6t
Soil Cd availability (mg/kg soil)																
0	9.8k	9.7l	9.5n	9.2q	9.5n	9.4o	9.1r	8.9t	9.4o	9.1r	8.7v	8.5w	9.0s	8.5w	8.1y	7.6z
4	10.9c	10.4f	10.2h	9.8k	10.3g	10.0i	9.5n	9.1r	9.7l	9.3p	8.9t	8.8u	9.3p	9.0s	8.4x	8.1y
8	11.4a	11.0b	10.8d	10.2h	10.8d	10.5e	10.0i	9.5n	10.2h	9.9j	9.4o	8.9t	9.6m	9.3p	8.9t	8.4x

* Similar lettered data in each parameter are not statistically significant ($P > 0.05$).

Table 3. Effect of Rice husk ash and Salicylic acid on soil microbial respiration (mg C-CO_2/kg soil)

Diesel oil (% (W/W)	Rice husk ash % (W/W): 0				2				4				8			
Salicylic acid (mmol/l)	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3
0	11.4r*	11.7p	11.8o	12.6i	11.7p	12.1m	12.4k	13.1e	12.1m	12.4k	12.6i	13.7b	12.5j	12.8g	13.2d	14.2a
4	11.1t	11.3s	11.7p	12.1m	11.3s	11.7p	12.1m	12.7h	11.8o	12.1m	12.4k	13.1e	12.1m	12.5j	12.9f	13.5c
8	10.1w	11.0u	11.3s	11.7p	11.0u	11.3s	11.8o	12.2l	11.3s	11.6q	12.0n	12.8g	11.6q	12.0n	12.4k	13.1e

* Similar-lettered data are not statistically significant ($P > 0.05$).

Table 4. Effect of Rice husk ash and Salicylic acid on plant Pb and Cd availability (mg/kg)

Diesel oil (% (W/W)	Rice husk ash % (W/W): 0				2				4				8			
Salicylic acid (mmol/l)	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3
Plant Pb availability (mg/kg soil)																
0	70.2i*	69.3j	68.1k	66.2m	69.1j	67.4l	66.5m	64.2o	67.4l	64.1o	62.1q	58.7t	65.5n	61.3r	59.4s	55.4u
4	76.8d	75.2e	74.4f	73.4g	75.4e	74.3f	73.2g	72.1h	74.8f	73.8g	72.4h	70.2i	73.3g	70.3i	66.4m	63.4p
8	79.2a	78.4	77.7c	75.4e	77.4c	76.7d	75.4e	73.1g	76.2d	75.1e	74.7f	72.7h	74.3f	72.7h	69.3j	65.1n
Plant Cd availability (mg/kg soil)																
0	9.1g	8.9h	8.8i	8.5l	8.9h	8.5l	8.4m	8.1p	8.5l	8.4m	8.1p	7.8r	8.4m	8.2o	8.1p	7.8r
4	9.5c	9.1g	8.9h	8.7j	9.2f	8.8i	8.6k	8.4m	8.7j	8.5l	8.3n	8.1p	8.6k	8.3n	8.2o	8.0q
8	9.8a	9.4d	9.1g	8.9h	9.6b	9.1g	8.9h	8.8i	9.3e	8.8i	8.6k	8.3n	8.9h	8.6k	8.5l	8.1p

* Similar lettered data in each parameter are not statistically significant ($P > 0.05$).

Table 5. Effect of Rice husk ash and Salicylic acid on bio-degradation of diesel oil in the soil (% (W/W))

Diesel oil (% (W/W)	Rice husk ash % (W/W): 0				2				4				8			
Salicylic acid (mmol/l)	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3
0	NM*	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
4	51.8t	52.7s	54.1q	55.3p	53.7r	54.8q	56.2o	57.9n	55.6p	57.3n	58.9m	59.4l	56.2o	58.9m	61.3k	53.7r
8	65.3j	67.4i	68.6h	70.3g	68.2h	71.7f	72.7e	75.4c	70.2g	71.5f	73.5d	76.2b	71.5f	73.4d	75.8c	77.4a

*NM: Not measured, ** Similar-lettered data are not statistically significant ($P > 0.05$).

Table 6. Effect of Rice husk ash and Salicylic acid on APX and POX enzyme activity (Unit/mg protein)

Diesel oil (% (W/W)	Rice husk ash % (W/W): 0				2				4				8			
Salicylic acid (mmol/l)	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3	0	1	1.5	3
APX enzyme activity																
0	18.4k*	18.2l	18.1m	17.9o	18.1m	18.1m	17.7q	17.5s	17.8p	17.4t	17.2u	17.0w	17.4t	17.1v	16.7x	16.4y
4	18.8g	18.6i	18.5j	18.3	18.5j	18.4k	18.1m	17.9o	18.2l	18.0n	17.6r	17.2u	17.9o	17.6r	17.2u	17.0w
8	19.8a	19.5b	19.4c	19.1e	19.3d	19.1e	19.0f	18.7h	18.7	18.4k	18.2l	18.1m	18.4k	18.2l	17.8p	17.5s
POX enzyme activity																
0	24.9f	24.6i	24.3l	24.0o	24.5j	24.1n	23.7t	23.2u	24.2m	23.7t	23.4t	23.1v	23.9p	23.5s	23.1v	22.8x
4	25.2c	25.1d	24.9f	24.6i	25.0e	24.7h	24.4k	24.2m	24.7h	24.2m	24.0o	23.4t	24.2m	24.0o	23.4t	23.0w
8	25.9a	25.5b	25.1d	24.9f	25.5b	25.2c	24.8g	24.7h	25.0e	24.7h	24.3l	24.1n	24.6i	24.4k	23.8q	23.5s

* Similar-lettered data are not statistically significant ($P > 0.05$).

The increase in soil pollution with diesel oil has been found to significantly elevate the activity of APX and POX enzymes. Our study's results indicate that increasing soil pollution with diesel oil from 0 % to 8 % (w/w) has resulted in an 11.8 % and 17.6 % increase in APX and POX enzyme activity, respectively. However, the use of rice husk ash and salicylic acid has had an adverse effect on APX and POX enzyme activity, suggesting that organic amendments can mitigate the impact of stress caused by heavy metals or petroleum compounds. To comprehend the effects of abiotic stresses, such as heavy metal toxicity, on plant physiological function and growth, monitoring the production of antioxidants in plants may prove useful. Hence, the presence of plant enzyme activity, such as APX or POX, can serve as an effective indicator of abiotic stressors, such as heavy metal or petroleum hydrocarbon pollution [50].

4. Conclusion

Our study has revealed that the application of rice husk ash has had a significant effect on reducing plant Pb and Cd concentrations. Increasing the application of rice husk ash from 0 % to 4 % and 8 % (w/w) has resulted in a significant decrease in plant Pb and Cd concentrations by 12.2 % and 16.7 %, respectively. On the other hand, increasing soil pollution with diesel oil from 0 % to 8 % (w/w) has significantly increased plant Pb and Cd concentrations by 13.1 % and 15.8 %, respectively. This increase can be attributed to the role of diesel oil in increasing soil salinity and, thereby, increasing the solubility of Pb and Cd in the soil. However, the impact of plant physiology on heavy metal uptake by plants cannot be ignored. Furthermore, both soil and foliar application of rice husk ash and salicylic acid have been found to have a significant effect on increasing the biodegradation of diesel oil in the soil. This effect can be attributed to the role of organic amendments in increasing soil microbial respiration and, consequently, increasing the bio-degradation of diesel oil in the soil. Nonetheless, further studies are required to investigate the role of soil physical and chemical characteristics, type of pollutants, and plant physiological characteristics in the biodegradation of petroleum compounds. Moreover, given the changing climatic conditions, the role of soil salinity and drought stress on the biological degradation of petroleum hydrocarbons in soil should also be taken into consideration.

Author Contributions

Amir Hossein Baghaie: The research conceptualization, data curation, formal analysis, funding acquisition, paper investigation, writing the methodology, project administration, software using, project supervision, data validation, writing the original draft, review, and paper editing. The Author read and approved the final manuscript.

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Conflicts of Interest

The Author declares that there is no conflict of interest.

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Ethical considerations

This article was approved by the Ethics Committee of Islamic Azad University, Arak Branch (IR.IAU.ARAK.REC.1398.019).

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