Chiang Mai J. Sci. 2023; 50(1): e2023008
https://doi.org/10.12982/CMJS.2023.008
Journal homepage : http://epg.science.cmu.ac.th/ejournal/

Research Article

Effect of Soil Amendments on the Enzymatic Profile of Soil when *Nicotiana alata* L. and *Petunia hybrida* L. were Irrigated with Synthetic Heavy Metal-contaminated Wastewater

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Received: 26 September 2022
Revised: 13 December 2022
Accepted: 19 December 2022

ABSTRACT

The nutrient cycle and organic matter decomposition are catalyzed by soil enzymes. In this study, enzymatic activities of catalase, dehydrogenase, alkaline phosphatase, and urease are studied in soils amended with compost (C), moss (M), or biochar (B) and irrigated with heavy metal-contaminated wastewater (HM-CW), when *Nicotiana alata* L. and *Petunia hybrida* L. was grown in pots. The irrigation of HM-CW reduced the soil enzyme activities. However, combined used of 5% M+C+B, results in the improved soil enzyme activities. In case of *N. alata* and *P. hybrida*, catalase activity was 222.03 ± 9.24 and 402.34 ± 10.48 mg $KMnO_4$ g^{-1} soil h^{-1}, respectively, with M+C+B, which was up to 94% higher than non-amended treatment. Similarly, the M+C+B treatment also showed higher activity for dehydrogenase i.e., 180.24 ± 6.95, and 156.79 ± 8.31 µg TPF g^{-1} soil h^{-1} for *N. alata* and *P. hybrida*, respectively, that were 73% and 49% higher than non-amended treatment. Alkaline phosphatase production (µg p-nitrophenol g^{-1} soil h^{-1}) for *N. alata* with M+C+B was 40.10 ± 1.92 and with C+B was 38.41 ± 2.00, while for *P. hybrida* with M+C+B was 39.33 ± 2.05, which is significantly higher as compared with the non-amended treatment. Urease activity at M+C+B application in soil with *P. hybrida* was 83.22 ± 5.54 mg urea g^{-1} soil h^{-1}, which was much higher than that of *N. alata*. In general enzyme activity enhanced in the soil with *N. alata* or *P. hybrida* along with soil amendments. It shows that application of these organic amendments individually or in combination with *N. alata* or *P. hybrida* increased enzyme activities possibly through affecting soil nutrient dynamics.

Keywords: *Petunia hybrida* L., *Nicotiana alata* L., soil amendments, catalase, dehydrogenase, urease, alkaline phosphatase

1. INTRODUCTION

One of the Earth's most intricate systems is soil, which is made up of biological, chemical, and physical elements [1]. The interactions between these elements are essential for the ecosystem's healthy operation [2]. Soil plays both a provisionary and regulatory role, ranging from nutrient recycling, carbon sequestration, nutrient provision, and waste degradation in maintaining the ecological balance [3, 4]. Various anthropogenic activities, such as land-use, mining, irrigation with wastewater, sludge application, and agrichemicals, negatively affect quality and functionality of soil [5, 6]. In recent decades, wastewater application has drawn the attention of scientists that elevated the concentrations of heavy metals (HMs) in the soils [7, 8]. About 20 million hectares of the global land is affected by HMs contamination [8, 9]. It is well known that persistent HMs disrupt the ecological balance of soils by altering the microbiome, physio-chemical properties, and the mobility of nutrients, but they are also hazardous once they enter the food chain, with the risk that enzyme specificity will change, cellular function can be disrupted, and the cell membrane and DNA structure could be damaged [10, 11]. This can eventually lead to systematic organ failures and even different cancers and tumors, in flora and fauna.

Soil health and food security are emerging challenges for the modern world, requiring more crop yield for the growing population with limited resources (clean water and air, and fertile soil) and time [2]. Soil contamination with HMs has further intensified this issue. To mitigate the soil contamination, various in-situ and ex-situ techniques are employed to find ways for soil reclamation such as excavation, application of chemicals (chelating agents including N, N-bis(carboxymethyl)-L-glutamic acid tetrasodium salt, chitosan, and ethylenediamine tetra acetic acid, nano-composite adsorbent), the addition of amendments (biochar, activated charcoal and moss), soil flushing, and phytoremediation [12, 13, 14]. Each approach has its limitations: the application time, resources, and human expertise needed [15, 16]. The complexity of soil is the result of biological and abiotic interactions. Physiological responses of plants when irrigated by wastewater can be improved by adding soil conditioners, including biochar, compost, and peat moss [7]. This addition can influence microbial populations and improve soil fertility and enzymes production [17, 18]. To some extent, the use of ornamental plants (OPs) for phytoremediation has gained attention in the recent years [19]. There is little work on soil enzymatic profiling with OPs and soil amendments [20, 21].

The application of soil amendments can influence the soil environment's response and enzymatic profile [17]. As these soil amendments affect the soil microbiome, the impact on the soil enzymatic profile is also evident. Soil enzymes were reported to be reduced due to the toxicity of petroleum hydrocarbons and HMs contaminated sites [22]. Different soil amendments like biochar, compost or lime addition, and sludge application have been used to enhance these enzymes' production, and promising results have been reported [23]. Biochar application has been reported to increase the adsorption of enzymes by providing a large porous surface area for enzymatic reactions and reducing HMs mobility through enhanced adsorption [24]. Compost supplies nutrients, humic acids, and promote microbial growth. It is also reported to improve soil fertility and promote crop growth [25]. Similarly, peat moss also provides nutrients to microorganisms and reduces metal bioavailability, thus fluctuates the soil's enzymatic status [7].

This experiment was conducted in continuation to the work reported by Khan et al. [7], where the plant physiological and biochemical status and metal compartmentalization in *Nicotiana alata* L. and *Petunia hybrida* L. were analyzed. However, the results for soil enzymatic profile under three different soil amendment application are

presented here. Therefore, the aim of this study is to evaluate the impact of different types of soil amendments (biochar, compost, and peat moss) on the soil's enzymatic profile (dehydrogenase, catalase, alkaline phosphatase, and urease) in the presence *N. alata* and *P. hybrida*. These enzymes are best indicator to assess the soil health during wastewater application [26]. Furthermore, the effects of individual and combined application of soil amendments along with *N. alata* and *P. hybrida* were assessed.

2. MATERIALS AND METHODS

A major part of the experiment includes a pot-based assessment of the enzymatic activity in contaminated soils treated with different organic amendments (biochar, compost, and peat moss). *N. alata* and *P. hybrida* were cultivated in pots, with and without soil amendments, and details of physiological responses are already presented by Khan et al. [7].

2.1 Physicochemical Analysis of Soil

The pH, electrical conductivity (EC), texture, and soil moisture were evaluated for the physicochemical analysis of soil. For pH measurement, 20 g of the soil was mixed with 20 mL of deionized distilled water and stirred well. After 30 min of resting, pH was recorded. The EC was measured with a conductivity meter in 2:10, soil-water solution. Water holding capacity was determined by adding distilled water to the 10 g soil sample, and the weight was recorded before and after the water was fully drained overnight. The difference in each weight was used to calculate water holding capacity. The hydrometer method was used to determined soil texture, as adapted by Mosaffaei et al. [27].

2.2 Cultivation of Plants

Commercially available seeds of the plant species, *N. alata* and *P. hybrida*, were purchased from Green Impex, Islamabad, Pakistan. The seeds were washed and potted in sterilized sand containing pots. Pots were incubated at 22 °C and watered regularly. Uniform seedlings were collected at two leaves stage after two weeks and shifted to pots having sterilized agriculture soil. Soil has a sandy loam texture with a pH of 8.2, EC 320 μS cm^{-1}, and moisture 62%. The detailed characteristics of soil are given in Table 1. The agricultural soil was collected from agricultural land located within Quaid-i-Azam University and was sterilized using wet autoclaving with Hirayama HVE-50 autoclave sterilizer (the autoclave sterilization was done at 121 °C for 20 min). For each pot to be used in

Table 1. Physiochemical characteristics of soil.

Parameters	Values
pH	8.2
EC	320 μS cm^{-1}
Moisture	62%
Texture	Sandy loam
Pb	0.001 mg kg^{-1}
Cd	0.05 mg kg^{-1}
Cr	0.001 mg kg^{-1}
Cu	0.46 mg kg^{-1}
Ni	0.06 mg kg^{-1}

the treatment, one plant was planted. During the experiments, containers were kept in the wire house under ambient conditions of 27/18 °C with a temperature difference between day and night. Light/dark photoperiods were 10/14 h with 65% relative humidity. In order to acclimate seedlings, the plants were watered twice daily with tap water. The moisture content in the soil was kept at soil field capacity for 5 weeks. The plants were then cultivated on synthetic wastewater irrigation with soil amendments, i.e., biochar (B), compost (C), and peat moss (M). After transplantation, respective treatments were watered with synthetic wastewater (details in section 2.3), and after 6 weeks of treatment, soil samples were collected from each treatment. Details related to the plant physiological response is already published [7].

2.3 Synthetic Wastewater and Experimental Setup

The pots were irrigated with synthetic wastewater with HMs composition for Ni 5 mg L^{-1}, Pb 2 mg L^{-1}, Cr 2.5 mg L^{-1}, Cu 5 mg L^{-1}, and Cd 2 mg L^{-1}. To prepare the synthetic water $N_2NiO_6.6H_2O$ for Ni, $Pb(NO_3)_2$ for Pb, $Cr(NO_3)_3$ for Cr, $CuSO_4.5H_2O$ for Cu, $CdCl_2.H_2O$ for Cd, were used. After six weeks of irrigation by synthetic effluents, plants were harvested. Each of the soil amendments (individual or combination) used in the experiments was taken as 5% of the total soil volume. Studied treatments for each plant were peat moss (M), compost (C), biochar (B), M+B, M+C, C+B, M+C+B. Plant control irrigated with contaminated water (CW) was also used. At the time of plant harvest, the soil samples of these two plants with different amendments were stored at -80 °C to analyze enzyme assay and prevent any deterioration or change in the condition.

2.4 Soil Enzymes Assay

2.4.1 Dehydrogenase activity

For dehydrogenase activity, 10 g of moistened soil was added in 10 mL triphenyl tetrazolium chloride (TTC) solution (5 g TTC in 0.2 M Tris-HCl buffer, pH 7.4) in a 50 mL plastic tube. The solution was kept in an incubator at 37 °C for 12 h. After that, two drops of concentrated H_2SO_4 were added to complete the reaction. The resultant solution was vortexed with 10 mL of toluene followed by shaking at 250 rpm on a rotary shaker for 30 min. After that, the solution was centrifuged at 4500 G for 5 min, and the red color supernatant triphenyl formazan (TPF) was obtained. The TPF contents of the sample solutions were calculated by comparing with standard curve plotted for standards of 25, 50, 75, 100, 125, 150, 175, and 200 μmol of TPF. The samples were analyzed at 400 nm on a spectrophotometer. Soil dehydrogenase activity was stated as μg TPF g^{-1} h^{-1} [28].

2.4.2 Catalase activity

Catalase activity was determined by following protocol adopted by Yang et al. [29], using back-titrating of residual H_2O_2 with $KMnO_4$. About 4 g of soil was mixed with 0.08 L of distilled and 0.015 L of 0.3% H_2O_2. The solution was well shaken for 35 min, and after that, 5 mL of 1.5 M H_2SO_4 was added. The solution was filtered and reacted with 0.02 M $KMnO_4$. Results were stated mg $KMnO_4$ g^{-1} soil h^{-1} by using the reacted amount of 0.02 M $KMnO_4$ utilized.

2.4.3 Alkaline phosphatase activity

Alkaline phosphatase was quantified according to the protocol of Jabborova et al. [30]. Briefly, 2 g of soil was poured in 500 μL of toluene and 8 mL of modified universal buffer (MUB) of pH 11. The MUB was prepared with 12.1 g of Tris, 11.6 g of maleic acid, 14 g of citric acid, and 6.3 g of boric acid in 0.5 L of 1 M NaOH. The solution was diluted to 1 L with distilled water, and 2 mL of p-nitrophenyl phosphate solution was added in soil suspension. The p-nitrophenyl solution consisted of 2.927 g disodium p-nitrophenyl phosphate in about 40 ml MUB and volume raised to 50 ml with the buffer. The resulting mixture was gently vortexed and incubated at

37 °C for 1 h. After 1 h, the soil was treated with 2 mL of calcium chloride (0.5 M) and 8 mL of sodium hydroxide (0.5 M). The yellow color suspension acquired was filtered through grade no. 2 filter paper. The samples were analyzed with a spectrophotometer at 400 nm. The p-nitrophenol content of the sample solution was measured by comparing with the standard curve plotted based on the standards of 0, 100, 200, 300, 400, and 500 ppb of p-nitrophenol, and their absorbance was measured at the wavelength of 400 nm. The results are stated as μg p-nitrophenol g^{-1} h^{-1}.

2.4.4 Urease activity

The urease activity in soil was measured by taking 1 g of soil in 50 mL of the conical flask, then 1 mL of urea solution (0.01 g urea mL^{-1}) was added and incubated at 37 °C for 5 h. After incubation, 10 mL of 2 M KCl solution with 5 mg L^{-1} phenylmercuric acetate was poured into the sample. Then this mixture was placed on a rotatory shaker for 1 h and filtered through Whatman filter paper 42. For the development of color, 2 mL of this filtrate was mixed with 2 mL of 2 M KCl-phenylmercuric acetate solution and 6 mL of coloring agent. The coloring agent was the solution of 10 mL of 0.25% thiosemicarbazide and 25 mL of 2.5% diacetylmonoxime in 0.5 L acid reagent (300 mL of 85% phosphoric acid and 10 mL of concentrated sulphuric acid, diluted up to 0.5 L with distilled water). The mixture obtained was placed for 30 min in a water bath and then in ice-cold water for 15 min. The urea content of the sample solution was determined by comparing it with the standard curve plotted based on standards; 0, 200, 400, 600, 800, and 1000 ppm of urea. The absorbance of the solution was determined at 527 nm, and urease activity is shown as μg urea hydrolyzed g^{-1} h^{-1} [31].

2.5 Statistical Analysis

Descriptive (mean, SD) and inferential (analysis of variance, multivariate analysis of variance, and posthoc test) statistical analysis was performed using IBM SPSS Statistics 21.0 software. To analyze the impact of individual and combination of the factors (soil amendments and plant type) on the enzyme activity, a two-way multivariate analysis of variance (MANOVA) was performed and presented in Table 2. The details related to the subject factors involved in the analysis are presented in Supplementary Table S1. To show the fitness of the two-way MANOVA model, the tests of significance for each model effect are presented in Supplementary Table S2. These tests included Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root. All these tests are positive-valued statistics. The higher/increasing values and significances indicate a good model fit, showing that amendments contribute more to the model. Hence, the null hypothesis can be rejected for large values.

3. RESULTS AND DISCUSSION

3.1 Dehydrogenase Activity

The results for soil dehydrogenase activity in *N. alata* and *P. hybrida*, respectively, with different treatments, are summarized in Figure 1. In the absence of plants in all the amendments, no significant differences were observed for dehydrogenase activity, while significantly lower activity was observed only in the case of soil (without any amendment). With *N. alata* enhanced dehydrogenase activity were observed with soil amendments compared to the non-vegetative treatments (Figure 1.a). In the case of no plant but with amendment M+C+B, significantly lower dehydrogenase activity of (117.33 ± 5.7 μg TPF g^{-1} soil h^{-1}) was observed compared to the plant. With *N. alata*, a significantly highest improvement in dehydrogenase activity was observed in soils amended with M+C+B (180.24 ± 6.95 μg TPF g^{-1} soil h^{-1}), which increased 58%, compared to same treatment without plant. It was 83% higher than treatment in which only *N. alata* was used without amendment, showing activity of 104.4 ± 3.95 μg TPF g^{-1} soil h^{-1}. *N. alata* with M+C+B showed up to 50, 48, 44, 44, 43, and 39% higher dehydrogenase

Table 2. Statistical analysis (Two-way MANOVA) for studied parameters and plants against enzyme activities.

Source of Variance	Variable[a]	Sum of Squares	df	Mean Square	F	Significance[b]
Moss	URE	115.004	1	115.004	25.979	0.000*
	DHA	10.614	1	10.614	0.213	0.646
	CAT	2.926	1	2.926	16.592	0.000*
	ALP	505.342	1	505.342	75.453	0.000*
Compost	URE	1857.831	1	1857.831	419.684	0.000*
	DHA	187.044	1	187.044	3.759	0.056
	CAT	69.187	1	69.187	392.313	0.000*
	ALP	18.910	1	18.910	2.823	0.097
Biochar	URE	1391.483	1	1391.483	314.336	0.000*
	DHA	5339.800	1	5339.800	107.309	0.000*
	CAT	14.046	1	14.046	79.644	0.000*
	ALP	280.493	1	280.493	41.881	0.000*
Plant	URE	38792.000	2	19396.000	4381.554	0.000*
	DHA	9754.416	2	4877.208	98.013	0.000*
	CAT	1110.876	2	555.438	3149.512	0.000*
	ALP	2628.659	2	1314.329	196.243	0.000*
Moss * Compost	URE	34.442	1	34.442	7.780	0.007*
	DHA	1432.090	1	1432.090	28.779	0.000*
	CAT	16.027	1	16.027	90.878	0.000*
	ALP	14.529	1	14.529	2.169	0.145
Moss * Biochar	URE	0.019	1	0.019	0.004	0.947
	DHA	5469.013	1	5469.013	109.906	0.000*
	CAT	5.192	1	5.192	29.441	0.000*
	ALP	1.327	1	1.327	0.198	0.658
Moss * Plant	URE	17.885	2	8.942	2.020	0.140
	DHA	2935.991	2	1467.995	29.501	0.000*
	CAT	1.048	2	0.524	2.970	0.058
	ALP	287.139	2	143.570	21.436	0.000*
Compost * Biochar	URE	399.084	1	399.084	90.153	0.000*
	DHA	2947.164	1	2947.164	59.226	0.000*
	CAT	51.652	1	51.652	292.886	0.000*
	ALP	5.748	1	5.748	0.858	0.357
Compost * Plant	URE	716.524	2	358.262	80.931	0.000*
	DHA	1050.702	2	525.351	10.557	0.000*
	CAT	55.675	2	27.838	157.848	0.000*
	ALP	72.298	2	36.149	5.397	0.007*

[a]URE= Urease, DHA= Dehydrogenase, CAT= Catalase, ALP= Alkaline phosphatase

[b]at significance level $p < 0.05$, while *indicates statistical significant difference

Table 2. (Continued).

Source of Variance	Variable[a]	Sum of Squares	df	Mean Square	F	Significance[b]
Biochar * Plant	URE	1545.840	2	772.920	174.603	0.000*
	DHA	2008.460	2	1004.230	20.181	0.000*
	CAT	26.376	2	13.188	74.780	0.000*
	ALP	78.151	2	39.076	5.834	0.004*
Moss * Compost * Biochar	URE	8.086	1	8.086	1.827	0.181
	DHA	4798.224	1	4798.224	96.425	0.000*
	CAT	11.802	1	11.802	66.919	0.000*
	ALP	61.630	1	61.630	9.202	0.003*
Moss * Compost * Plant	URE	15.447	2	7.723	1.745	0.182
	DHA	6014.011	2	3007.005	60.429	0.000*
	CAT	78.883	2	39.442	223.646	0.000*
	ALP	35.518	2	17.759	2.652	0.077
Moss * Biochar * Plant	URE	10.891	2	5.445	1.230	0.298
	DHA	4657.227	2	2328.614	46.796	0.000*
	CAT	0.651	2	0.325	1.845	0.165
	ALP	126.920	2	63.460	9.475	0.000*
Compost * Biochar * Plant	URE	955.072	2	477.536	107.875	0.000*
	DHA	8097.960	2	4048.980	81.369	0.000*
	CAT	88.478	2	44.239	250.851	0.000*
	ALP	48.204	2	24.102	3.599	0.032*
Moss * Compost * Biochar * Plant	URE	385.433	2	192.717	43.535	0.000*
	DHA	4711.468	2	2355.734	47.341	0.000*
	CAT	22.541	2	11.271	63.908	0.000*
	ALP	41.714	2	20.857	3.114	0.050
Residual	URE	318.725	72	4.427		
	DHA	3582.789	72	49.761		
	CAT	12.698	72	0.176		
	ALP	482.216	72	6.697		

[a]URE= Urease, DHA= Dehydrogenase, CAT= Catalase, ALP= Alkaline phosphatase

[b]at significance level $p < 0.05$, while *indicates statistical significant difference

activity as compared to M, C, M+C, C+B, B, M+B, and C+B, respectively. The impact of soil amendment on soil cultivated with and without *P. hybrida* is shown in Figure 1.b. The overall trend of enzymatic activity was similar to that of *N. alata*. Significantly highest dehydrogenase activity was noted for M+C+B amended soils with *P. hybrida,* which was 156.79 ± 8.31 μg TPF g^{-1} soil h^{-1}, which was 49% higher than the non-amended treatment with an activity level of 105.10 ± 2.66 μg TPF g^{-1} soil h^{-1}. *P. hybrida* with all other treatments, show improved dehydrogenase activity compared to unamended treatment. However, a significant difference was not observed among the amended treatment, except in the case of M+C+B.

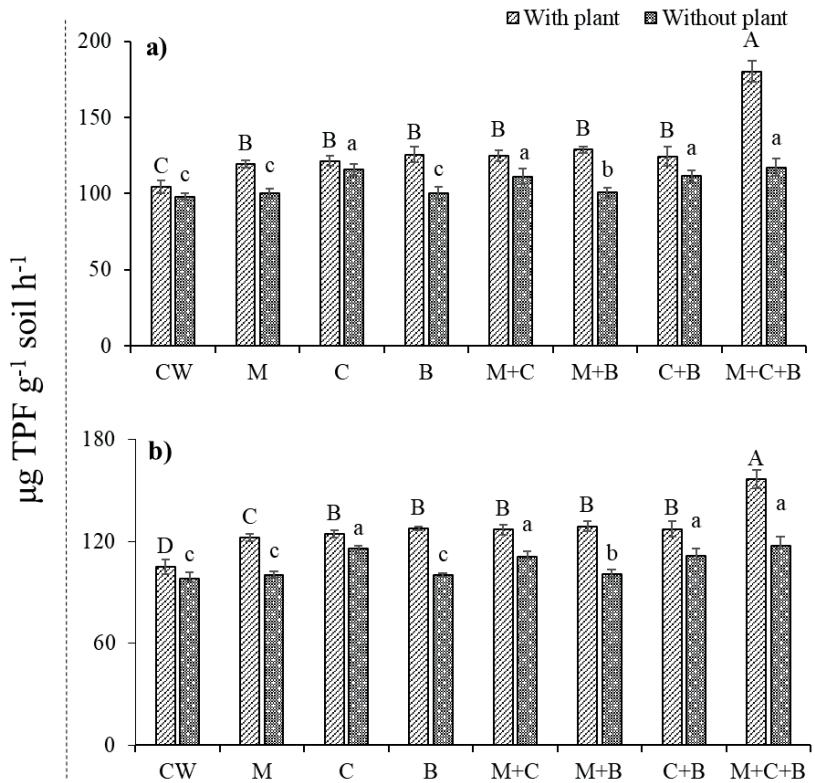

Figure 1. Dehydrogenase activity in soil with **a)** *N. alata* and **b)** *P. hybrida* irrigated by synthetic wastewater with amendments, moss (M), compost (C), biochar (B), (M+C), (M+B), (C+B), (M+C+B), and control treatment irrigated with contaminated water with no amendment (CW). The number of observations were 3. Bar denoted with different alphabets were significantly different at significance level $p < 0.05$ according to Duncan test.

3.2 Catalase Activity

Catalase activity in soils with and without *N. alata* and *P. hybrids* is shown in Figure 2. A significant increase in the enzymatic activity was observed in case of soil amendments with plants compared to non-planted treatment. *N. alata* with M+B+C showed significantly higher production of catalase enzyme, i.e., 222.03 ± 9.24 mg $KMnO_4$ g^{-1} soil h^{-1}, than the other amendments (Figure 2.a). There was a significantly higher difference between M, C, B, M+C, M+B, and B+C compared to CW in the non-planted treatments. However, the highest increment was noted for C+B and M+B+C. *P. hybrida* with M+C+B showed significantly highest catalase production (402.34 ± 10.48 mg $KMnO_4$ g^{-1} soil h^{-1}), which was 94% higher than the non-amended with plant treatment (Figure 2.b). In the absence of plants, the amendments of C+B and M+C+B showed significantly higher catalase activity.

3.3 Alkaline Phosphatase Activity

Soil alkaline phosphatase activity showed variable results with the application of different amendments. All the amendments in the presence or absence of plants showed higher alkaline phosphatase

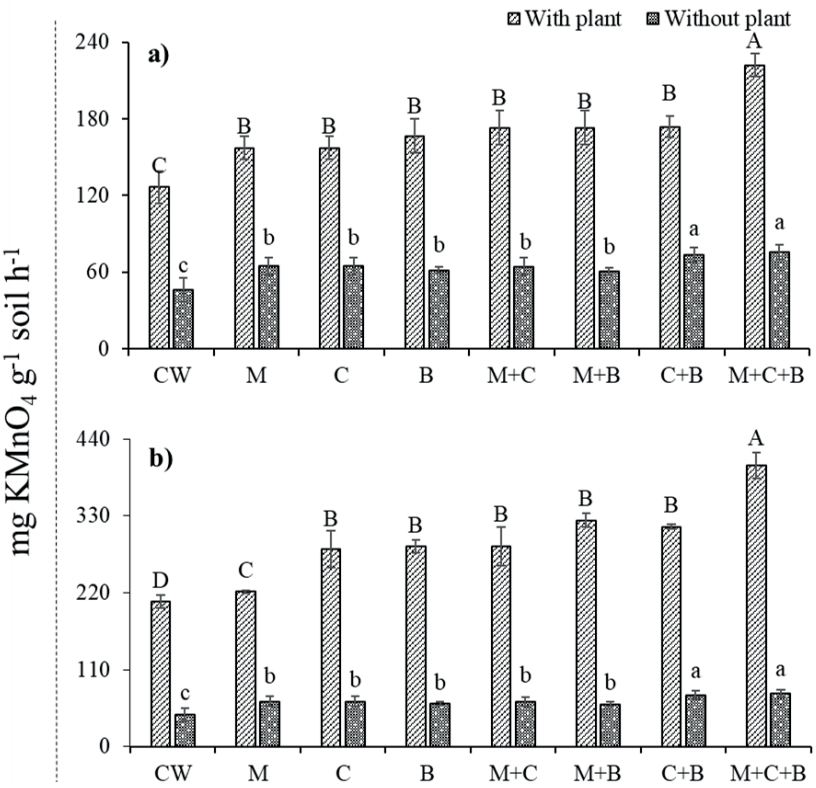

Figure 2. Catalase activity in soil with **a)** *N. alata* and **b)** *P. hybrida* irrigated by synthetic wastewater with amendments, moss (M), compost (C), biochar (B), (M+C), (M+B), (C+B), (M+C+B), and control treatment irrigated with contaminated water with no amendment (CW). The number of observations were 3. Bar denoted with different alphabets were significantly different at significance level $p < 0.05$ according to Duncan test.

activity than control (CW) treatment. *N. alata* with M+C+B (40.10 ± 1.92 µg p-nitrophenol g^{-1} soil h^{-1}) and C+B (38.41 ± 2.00 µg p-nitrophenol g^{-1} soil h^{-1}) resulted in the maximum alkaline phosphatase activity by 37 and 32% greater than CW, respectively (Figure 3.a). In the case of no plant, co-amendment of M+C+B significantly increased the production of alkaline phosphatase enzyme, i.e., 34.96 ± 3.34 µg p-nitrophenol g^{-1} soil h^{-1}, compared to other treatments except C+B (Figure 3.a). The lowest alkaline phosphatase activity (30.79 ± 1.72 µg p-nitrophenol g^{-1} soil h^{-1}) was observed in soil without any amendment (CW treatment) for *N. alata*. In case of *P. hybrida* with M+C+B treatment, significantly higher production of alkaline phosphatase enzyme was observed (39.33 ± 2.05 µg p-nitrophenol g^{-1} soil h^{-1}), compared to all other treatments (Figure 3.b).

3.4 Urease Activity

Soil urease activity showed variable results with the application of a different combination of amendments. *N. alata* with M+C+B showed significantly increased production of urease activity, i.e., 28.45 ± 1.24 mg urea g^{-1} soil h^{-1} compared to other amendments, followed M+C and C+B

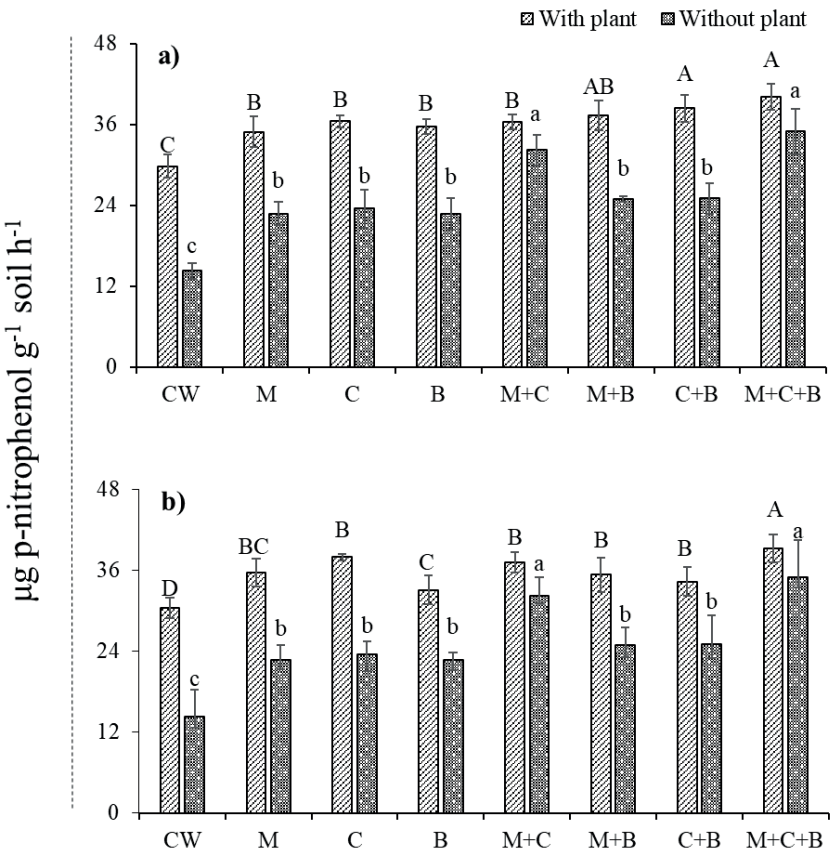

Figure 3. Alkaline phosphatase activity in soil with **a)** *N. alata* and **b)** *P. hybrida* irrigated by synthetic wastewater with amendments, moss (M), compost (C), biochar (B), (M+C), (M+B), (C+B), (M+C+B), and control treatment irrigated with contaminated water with no amendment (CW). The number of observations were 3. Bar denoted with different alphabets were significantly different at significance level $p < 0.05$ according to Duncan test.

statistically higher to other treatments and control (Figure 4.a). Similarly, with *P. hybrida*, the M+C+B application in soil showed significantly higher production of urease, i.e., 83.22 ± 5.54 mg urea g^{-1} soil h^{-1} compared to other amendments (Figure 4.b). In the absence of plants, the highest activity was observed for M+C, C+B, and M+B+C showed significantly higher urease activity, 22.70 ± 1.42, 22.39 ± 1.07, and 21.38 ± 1.96 mg urea g^{-1} soil h^{-1}, respectively for the corresponding treatments, as compared to the unamended treatment (Figure 3.b).

3.5 Individual and Interactive Effects of Amendments and Plants on Soil Enzymes

Among the studied combinations were moss, compost, biochar, plant, moss + compost, moss + biochar, moss + plant, compost + biochar, compost + plant, biochar + plant, moss + compost + biochar, moss + compost + plant, moss + biochar + plant, compost + biochar + plant, and moss + compost + biochar + plant, against dependent variable of soil enzyme activity. Most of the individual and interactions of the applied independent variable

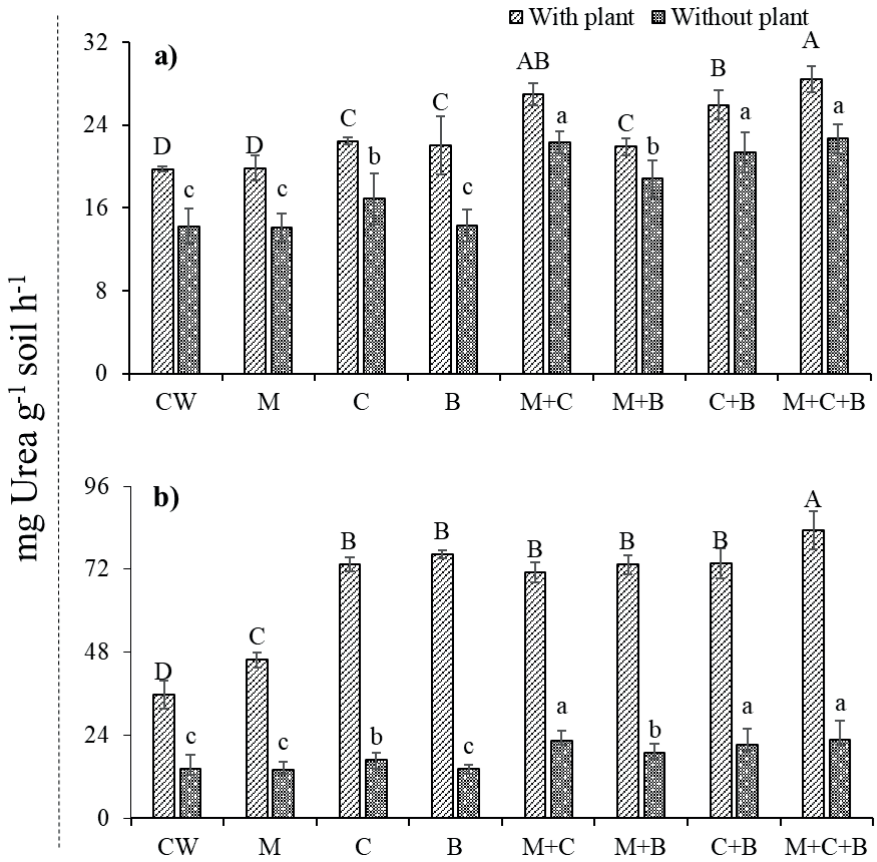

Figure 4. Urease activity in soil with **a)** *N. alata* and **b)** *P. hybrida* irrigated by synthetic wastewater with amendments, moss (M), compost (C), biochar (B), (M+C), (M+B), (C+B), (M+C+B), and control treatment irrigated with contaminated water with no amendment (CW). The number of observations were 3. Bar denoted with different alphabets were significantly different at significance level $p < 0.05$ according to Duncan test.

of soil amendment and plant were significant (at significance level $p < 0.05$). The individual effect of moss on dehydrogenase and compost on dehydrogenase and alkaline phosphatase was found insignificant. While the combined effect of moss + compost on alkaline phosphatase, moss + biochar on urease and alkaline phosphatase, moss + plant on urease and catalase, compost + biochar on alkaline phosphatase, moss + compost + biochar on urease, moss + compost + plant on urease and catalase, and moss + biochar + plant on urease and catalase were noted to be insignificant.

3.6 Discussion

Soil HM contamination affects the soil enzymatic activity as this contamination is recognized to negatively affect the microbial community structure and availability of nutrients for plants and microorganisms [32]. The present work was focused on assessing the effect of soil amendments (peat moss, compost, and biochar)

on the enzymatic profile of soil planted with *N. alata* and *P. hybrida* when irrigated with synthetic wastewater contaminated with HMs (Ni, Pb, Cr, Cu, and Cd). Secondly, the impacts of individual and co-amendments along with the type of plants on soil enzymatic profile were also investigated that can alter the enzymatic profile. The soil experiments conducted by Khan et al. [7] were used to test this hypothesis, where soil enzyme activities were analyzed in soil from different treatments. These soil enzymes included dehydrogenase, catalase, alkaline phosphatase, and urease activity.

Among the previously reported studies, it is well established that changing the soil microbial and nutrients composition alter the enzyme activities. Further, metal(liod)s contaminated soil show variability in enzyme activities [33]. Hence, the HMs can have positive or negative implications depending on the enzyme activities. The HMs are known to cause hindrance in C, N, P, S, and other nutrient cycling in soil [26]. One of the potent ways to study the C cycling in the soil is the dehydrogenase activity. It is responsible for the hydrogenation of inorganic acceptors using organic compounds [34]. Improvement in the dehydrogenase enzyme activity is a positive indication linked with improved microbial activity and nutrient cycling [35]. The use of sugarcane bagasse biochar, compost, and moss has been shown to improve the dehydrogenase activity in soil [36, 37]. Similar findings were noticed in this study where soil amended with compost, moss, and biochar (individually and in combination) have demonstrated an increment in the dehydrogenase activity up to 73% compared to the non-amended treatment (Figure 1.a and 1.b, and Table 2). This improvement in soil dehydrogenase activity is due to co-application of organic amendments that reduce the metal availability and protein denaturation. In contrast, it increased soil microbial diversity and improved soil quality [38]. The impact of different plant species was also evident on the soil dehydrogenase activity, as differences were observed between *N. alata* and *P. hybrida* (Table 2). This might be due to the plant's crucial roles in maintaining a balanced microbial population in the rhizosphere. These roles include providing food and shelter to microorganisms, and in return the microbial population alter soil enzyme activities [39].

Catalase activity results from metabolic and respiratory activities of living organisms and releases cytotoxic hydrogen peroxide (H_2O_2) into water and oxygen [40]. This activity is very closely related to microbial activity. It also prevents reactive oxygen species (ROS) toxicity to the organism, which is generated due to hydrogen peroxide [25]. The soil catalase activity is directly linked with the soil biological status, compared to HMs which affect indirectly [33]. Kuscu et al. [40] also proposed that the catalase enzyme is significantly correlated with organic matter, calcium carbonate, and K levels. The present study showed similar findings where soil amendments (M+C+B) improved the soil catalase level. However, the impact of both studied plants (*N. alata* and *P. hybrida*) was more pronounced (Figure 2 and Table 2). Treatments with the studied plants and M+C+B significantly improved organic matter levels, which in turn facilitated the soil microbial population, leading to higher catalase activity.

Alkaline phosphatase is reported to be reduced when a soil is contaminated with HMs [32]. In principle, alkaline/acid phosphatase catalyzes the hydrolysis of ester phosphate bonds that releases inorganic phosphate (P) that is incorporated by microorganisms and plants [41]. Hence alkaline and acid phosphatase play a significant role in P cycling. Alkaline phosphatase activity significantly increased with plant and soil amendments (Figure 3). The cultivation of *N. alata* and *P. hybrida* also showed improved alkaline phosphatase activity, which could be due to enhanced rhizosphere activity (including organic matter content and physiochemical parameters) compared to the CW. This is because the plant roots and soil amendments improved the soil microbial population status. Hence, the combined

application of plants (*N. alata* and *P. hybrida*) with any of the soil amendments showed improved soil alkaline phosphatase activity (Table 2).

The HMs contamination negatively impact the urease level in soil [33]. The soil without any amendment had the least urease activity in the present study, while individually and combined soil amendments (biochar, compost, and moss) improved urease levels (Figure 2, Table 2). Similar results were reported by Liu et al. [8] with green waste compost on Pb-polluted soil on which *Brassica campestris* L. was cultivated. At the same time urease is an important indicator of soil fertility as it mediates the N cycle by generating plant bioavailable N, by catalyzing the hydrolysis of urea [26]. Hence, if the soil has higher organic matter, the reduced urease activity can reduce available N to plant, leading to decreased growth. Irfan et al. [35] reported that applying pyrolyzed organic carbon (biochar) to organic carbon deficient arid soil could improve the status of urease activity. Hence the use of soil amendment/s (like peat moss, compost, and biochar) and plants species are vital for the soil fertility concerning urease. In the present study, the addition of organic amendments like M+C+B along with *N. alata* and *P. hybrida* resulted in significantly higher urease production compared to individual amendment of C, B, and M.

The development of plants and the fertility of the soil are both greatly influenced by soil enzymes. The main sources of soil enzymes include soil fauna, underground plant components, and microorganisms. Information on biochemistry can be gleaned from an examination of soil enzyme activity [42]. Hence, the studies should be designed after making do consideration, on the above-mentioned factors. Further, screening of other available and commonly used ornamental plants, soil amendments, and modern advance composts and absorbent is necessary, for an efficient removal of metal(liod)s and improvement in the quality of the contaminated environments, notably soil [43, 44, 45]. Soil enzymes can help as a bioindicator for soil pollution regarding the HMs and other contaminants [18]. As the response of soil enzyme varies with reference to the type of contaminants, it will be very fruitful to assess and quantify such impact, to establish soil enzyme as a bioindicator for soil fertility and plant and microbial growth [19, 46]. To our understanding, this is one of the few studies conducted on soil where ornamental plants like *N. alata* and *P. hybrida* were grown. Soil is a non-renewable resource that supports the growth of many living organisms mostly autotrophs, so measures should be taken to conserve it [47]. A better understanding of its complex interaction can help the sustainable utilization of this depleting natural resource.

4. Conclusions

The influence of irrigation with heavy metal-contaminated wastewater on the soil enzymatic profile of *N. alata* and *P. hybrida* was examined in the current study. The application of soil amendment (M, C, B) greatly boosted the activity of soil enzymes. The combined use of M+C+B produced the most pronounced favorable condition. The cultivation of *N. alata* and *P. hybrida* showed 53% and 33% higher DHA activities, as compared to soil with only M+C+B amendment. Similar trend for the improvement of 14% and 12% for ALP activity with co-application of M+C+B with *N. alata* and *P. hybrida,* respectively, was noted. For urease activity *N. alata* showed an increment of 25%, while with *P. hybrida* a 266% higher URE activity was noted with M+C+B. The CAT activity was highest with combined use of amendments and plants, that was 190% and 430% higher than soil with M+C+B without plants. The analysis of the soil enzymatic profile demonstrated the improvement in enzyme activities due to the individual and combined interactions between different types of plants and soil additives. Specifically, the M+C+B soil co-amendment with the plants improved the enzymatic profile of the soil. The study suggests that nutrient-rich soil, along with proper plantation, can reduce the

toxic effects of heavy metals. Further, it can be concluded that some enzymatic activities of the soil can be improved by addition of amendments, as standalone option. However, co-cultivation of *N. alata* and *P. hybrida* can positively affect enzymatic status.

Acknowledgments

This research has been funded by Scientific Research Deanship at University of Ha'il - Saudi Arabia through project number RG-21 105.

Conflicts of interest

The authors declare no conflicts of interest.

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