Effects of Heavy Metals on Plant Growth and Metal Accumulation in Moringa Plant.

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Abstract

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Moringa oleifera sprung in soil contaminated with Lead and Magnesium. Stress of heavy metals of different strength was applied in each replicate. Physical parameters are necrosis, chlorosis, and height of plant included in this research. The sample Leaves, roots and stems are collected from each replicate after a week, washed, dried weighed, crushed, digested with Aqua Regia and at the end filtered. The extract was diluted to a specific extent with distilled water and quantitatively measured with Atomic Absorption Spectrophotometer. The data collected was statistically analyzed. Measured the Toxicity level and optimum conditions for plant growth and yield was calculated from the data collected. .

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1. Introduction

Moringa oleifera is found in most of countries. It is famous because it is more beneficial for us. Average height of Moringa oleifera is about 8m [1]. Its specialty is that it can grow up in any type of environment. It's also called 'sohanjana' [2]. It is mostly use in medicines in England and USA, but in Pakistan there is some work on it. It is exposed through study that all part of Moringa plant i.e. leaves, roots, stems, flowers and seeds used for treatment of diseases [3].

By commercializing the Moringa oleifera we can get many useful medicines for different diseases [4]. For water purification we can use its seeds. Ben oil also used as lubricating oil in Rado watches. Recently biofuel made from it through some scientists, Kenya is mostly using it in field [5]. It can be used as bio pesticide [6]. Heavy metal pollution is an important environmental pollution in the world [7].

In comparison with organic matters heavy metals cannot be transformed by microorganism and accumulate in soil, water, bottom sediments and living organisms [8]. Mostly heavy metals pollutants are present in the environment due to human activities [9]. The heavy metals Magnesium, Lead and iron cause a deleterious effect in plants, animals and human being [10]. The heavy metals can cause to change of photosynthetic cell, chlorophyll production, and pigment synthesis and enzyme activities [11]. Heavy metals from waste and industrial effluents cause harmful effect to plants [12]. Concentration of heavy metals mostly effected the growth of plants via different way. Different heavy metals effect differently at growth of plants [13]. In contaminated soil mostly plants growth reduces due to reduction of photosynthetic activities and little bit enzyme activities [14].

The aim of research is to assess the tolerance index of Pb and Mg and better understanding of heavy metals and toxicity in Moringa oleifera to maintain ecological harmony of our planet. The second perspective of research paper is to find defense response of Moringa plant. The interaction of heavy metals and plants has two aspects. One is negative effect of heavy metals on

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plants cause overall growth retardation and second is, some plants have their own defense response against heavy metals and detoxify heavy metal contaminant [15].

2. Experimental

2.1 Chemicals

Chemicals and salts used during research work were hydrochloric acid (HCl), nitric acid (HNO₃), magnesium sulphate (MgSO₄.7H₂O) and lead nitrate (Pb(NO₃)₂). All the chemicals and salts were of analytical grade and were used as such without any prior treatment.

2.2 Method

The experiment was started with purchasing of Moringa plant seed. The seed was purchased from nice seed (Seed supplier) Roshan center Allama Iqbal Town, Multan road Lahore. The soil was taken from our own field Awan Muslim Muridke Sheikhpura. The seedling was initiated after 7 days. The pots were filled with 3/4th part of each pot, leaving 1/4th part left. The water was showered after one day. Fifteen pots ready individually and transferred the Moringa plant when it is almost 4-5 inches.

Samples were collected very carefully to avoid any contamination. The collected sample of plants were sealed in polythene bags and tagged accordingly. These samples were then carried to the laboratory for further procedure. Roots, leaves and Stems were first washed with dil. HCl and then distilled H₂O. The Stems, leaves and roots were placed one day for sun dry, properly labelled and placed for 2 days in sun for maximum loss water contents. Drying of samples has been done in furnace at 120°C for 2-3 hours. All the water content evaporates and dried sample were grinded manually with the help of pestle and mortar to fine powder and then stored in polythene bags to avoid any dust contamination. Aqua regia had formed by mixing of HNO₃ and HCL 1:3 respectively for the process of digestion.

Digestion of sample had done in aqua regia for 48 hours. To each digested sample, 40 ml distilled H₂O was added to make total volume of 50ml. Last part of digestion process, each diluted sample was filtered with Whatman filter paper. The filtrate collected and stored in 120 ml bottles, lid, properly labelled and stored.

2.3 Elemental Standard Solution Preparation

1.6 g Pb(NO₃)₂ is dissolved in sufficient amount of distilled water so that total volume of solution will be one liter to get 1000 mg/L Pb solution. 10.14g

MgSO₄.7H₂O is dissolved in sufficient amount of distilled water so that total volume of solution will be one litre to get 1000 mg/L Pb solution. Dilute solution of 50 mg/L, 100 mg/L and 150 mg/L was prepared from standard solutions.

3. Results and Discussion

3.1 Effect of Lead

Absorbance of Lead in different parts of Moringa plant after first week was evaluated. And Absorption of lead was estimated in all parts of plant body (Roots, leaves, stem). The estimated absorbed amount in Roots, leaves and stem were increase by increasing the concentration of solution 0, 50, 100 and 150 mg/L. In first week, absorbance value of lead in leaves were the below detection limit of Atomic Absorption Spectrophotometer (AAS).

The maximum absorption in all four strengths was observed in roots and minimum absorption was in leaves. Descending order of Pb accumulated in different parts is: Roots < Stems < Leaves. Absorbance values of lead increased by increasing the concentration of lead in solution.

The accumulation of lead in roots at 0mg/L, 50mg/L, 100mg/L and 150mg/L of concentration were 0.017mg/L, 1.89mg/L, 1.93mg/L and 1.99mg/L respectively. The accumulation of lead in stem at 0mg/L, 50mg/L, 100mg/L and 150mg/L of concentration were BDL, 1.64mg/L, 1.75mg/L and 1.83mg/L respectively. But the accumulation of lead in leaves at all four concentrations was below detection limits of AAS.

3.2 Statistical Analysis

Student T tests statistical analysis of different parts of Moringa oleifera plant against different concentration of lead after first week is shown in table 1.

Two methods, one is accepted method and other is test method. A series of replicate measurements on a single sample using two methods can be done if accepted value is not available.

3.3 H₀ or Null hypothesis

If $t > t_v$ then the estimated heavy metal absorption by plant, the error in the experiment is not occurred by chance or happened determinate error. The determinate error may be instrumental AAS e.g., confirmation of metal, lamp position, lamp obstruction, Rescale, instrumental zero, optimize lamp and signal. The determinate error may quite possible due to inaccurate sample preparation, sample grinding, sample weighing and error in acid digestion process.

3.4 H1 hypothesis

If $t < t_v$ then the error in the experiment is occurred by chance. Error is not determinate. The indeterminate error may be present but not large enough to reject H1 hypothesis or test performed. If $t > t_v$ H1 hypothesis may or may not be rejected but the value viewed as suspected value. The rejection of suspected value can be tested by Q test. If $Q < Q_{95}$ even $t > t_v$ (suspected value) then the test is not rejected, error in the experiment may be occurred indeterminate.

Absorbance of Lead in different parts of moringa plant after second week were evaluated and the absorption of lead was estimated in all parts of plant body (Roots, leaves, stem). The estimated absorbed amount in Roots, leaves and stem were increase by increasing the concentration of solution 0, 50, 100 and 150mg/L. In second week, absorbance values in leaves were detected at 100 and 150mg/L concentration of solution.

Maximum absorbance of lead (Pb) is 2.45 mg/L when the concentration of solution is 150 mg/L. The absorbance value increase in roots, stem and leaves by increasing the concentration of solution but more in roots as compared to stem. Absorbance values in leaves were observed when used the concentration of 100 mg/L and 150 mg/L.

The maximum absorption in all four strengths was observed in roots and minimum absorption was in leaves. Descending order of Pb accumulated in different parts is: Roots > Stems > Leaves. Absorbance values of lead increased by increasing the concentration of lead in solution. The accumulation of lead in roots at 0mg/L, 50mg/L, 100mg/L and 150mg/L of concentration were 0.023mg/L, 2.13mg/L, 2.32mg/L and 2.45mg/L respectively. The accumulation of lead in stem at 0mg/L, 50mg/L, 100mg/L and 150mg/L of concentration were 0.013 mg/L, 1.73mg/L, 1.87mg/L and 1.95mg/L respectively. The accumulation of lead in leaves at 0mg/L, 50mg/L, 100mg/L and 150mg/L of concentration were BDL, BDL, 1.45mg/L and 1.75mg/L respectively.

3.5 Statistical Analysis

Statistical analysis of different parts of Moringa oleifera plant against different concentration of lead after second week is shown in table 2.

T test calculated in 50 mg/L, 100 mg/L and 150 mg/L Pb Stem were 4.382, 4.382 and 4.332 respectively. Similarly, 150 mg/L Pb roots calculated t value was 4.332, greater than tabulated value t_v . (Ref: Table 2).

The suspected values are not too much significantly different from tabulated value (4.303).

The suspected values in each case can be tested by Q test. If Q test tested that the suspected values are within the range and each value is not laid outside of the range, then result cannot be rejected.

The suspected results can be depicted as accidental error. The suspected value increases the standard deviation and variance which alter mean. Q test is one of all the test, range within significant observation fall is established if the range is too small then the good data can be rejected but if the range is too large the retention of erroneous measurements is maximum and suspected results can be rejected if Q value is equal to or greater than tabulated value.

50 mg/L Pb stem and 150 mg/L Pb roots, the suspected values were lie within the range in the Q test ($Q < Q_{95}$). Q values were 0.67 and 0.60 respectively. Similarly, 100 mg/L and 150 mg/L Pb stem, Q values were 0.33 and 0.60 respectively. The suspected values are less than the tabulated value ($Q < Q_{95}$) consequently 95% probabilities that suspected values are not outlier of the range, so results are not rejected (Ref: Table 3).

Absorbance of Lead (Pb) by different parts of Moringa oleifera plant against different concentration after third week were evaluated and the maximum concentration of lead accumulated in roots, Stems and leaves were 2.92 mg/L, 2.33 mg/L and 1.94 mg/L respectively in 150 mg/L Pb. The accumulation of lead at 0 mg/L is below detection limit of AAS. In controlled experiment 0 mg/L Pb, the absorption of Lead (Pb) in roots and Stems were 0.027 and 0.017 respectively but below detection level at concentration of 0 mg/L Pb solution in leaves. The absorption of Pb was increased in 50 mg/L Pb and 100 mg/L Pb. The accumulation of Lead (Pb) in roots, Stems and leaves in 50 mg/L Pb were 2.473 mg/L, 1.95 mg/L and 1.063 mg/L respectively. Similar estimated concentration of Lead (Pb) in 100 mg/L Pb in roots, Stems and leaves were 2.773 mg/L, 2.14 mg/L and 1.73 mg/L respectively. In 150 mg/L Pb, the maximum absorption of Lead in roots, Stems and leaves were 2.92 mg/L, 2.327 mg/L and 1.94 mg/L respectively.

3.6 Statistical analysis

Student T tests statistical analysis of different parts of Moringa oleifera plant against different concentration of lead after third week is shown in table 4. t test calculated in 50 mg/L Pb Stems and leaves were 4.379 and 4.332. Similarly, in 100 mg/L Pb roots and leaves, calculated t value was 4.309 for both, greater than tabulated value t_v 150 mg/L Pb Stems calculated t value was 4.312 (Ref: Table 4)

Table. 1 Statistical analysis of different parts of Moringa oleifera plant against different concentration of lead after first week.

Concentration (ppm)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t _v)	H ₁ rejection (t < t _v)	H ₀ rejection (t > t _v)
0	Roots	0.023±0.006	0.014	4.141	4.303	<4.303	
	Stems	0.013±0.006	0.014	4.141	4.303	<4.303	
	Leaves	BDL	0.000				
50	Roots	2.130±0.010	0.025	4.303	4.303		
	Stems	1.733±0.015	0.038	4.382	4.303		>4.303
	Leaves	BDL	0.000				
100	Roots	2.327±0.012	0.029	4.139	4.303	<4.303	
	Stems	1.877±0.015	0.015	4.382	4.303		>4.303
	Leaves	1.450±0.010	0.025	4.303	4.303		
150	Roots	2.453±0.025	0.063	4.332	4.303		>4.303
	Stems	1.953±0.025	0.063	4.332	4.303		>4.303
	Leaves	1.750±0.010	0.025	4.303	4.303		

Table. 2 Statistical analysis of different parts of Moringa oleifera plant against different concentration of lead after second week.

Concentration (ppm)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t _v)	H ₁ rejection (t < t _v)	H ₀ rejection (t > t _v)
0	Roots	0.017±0.006	0.014	4.141	4.303	<4.303	
	Stems	BDL	0.000				
	Leaves	BDL	0.000				
50	Roots	1.890±0.01	0.025	4.303	4.303		
	Stems	1.64±0.01	0.025	4.303	4.303		
	Leaves	BDL	0.000				
100	Roots	1.927±0.012	0.029	4.139	4.303	<4.303	
	Stems	1.75±0.01	0.025	4.303	4.303		
	Leaves	BDL	0.000				
150	Roots	1.990±0.010	0.025	4.303	4.303		
	Stems	1.83±0.012	0.029	4.139	4.303	<4.303	
	Leaves	BDL	0.000				

Table. 3 Q test Rejection test of suspected values of different parts of plant of various concentration of lead after second week

Concentration (mg/L)	Plant parts	H ₁ rejection (t > t _v)	Ranges of replicates (mg/L)			Difference between largest to nearest neighbor value	Difference between largest value and smallest value	Rejection Quotient at probability 95% (Q ₉₅)	Q < Q ₉₅
50	Stem	>4.303	1.72	1.73	1.75	0.02	0.03	0.97	0.67
100	Stem	>4.303	1.86	1.88	1.89	0.01	0.03	0.97	0.33
150	Stem	>4.303	1.93	1.95	1.98	0.03	0.05	0.97	0.60
	Roots	>4.303	2.43	2.45	2.48	0.03	0.05	0.97	0.60

Table 4. Student T tests statistical analysis of different parts of *Moringa oleifera* plant against different concentration of lead after third week.

Concentration (ppm)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t_v)	H_1 rejection ($t < t_v$)	H_0 rejection ($t > t_v$)
0	Roots	0.027±0.012	0.029	4.140	4.303	<4.303	
	Stems	0.017±0.006	0.014	4.141	4.303	<4.303	
	Leaves	BDL	0.000				
50	Roots	2.473±0.059	0.146	4.272	4.303	<4.303	
	Stems	1.950±0.026	0.066	4.379	4.303		>4.303
	Leaves	1.063±0.025	0.063	4.332	4.303		>4.303
100	Roots	2.773±0.055	0.137	4.309	4.303		>4.303
	Stems	2.140±0.020	0.050	4.303	4.303		
	Leaves	1.730±0.036	0.090	4.309	4.303		>4.303
150	Roots	2.920±0.046	0.114	4.286	4.303	<4.303	
	Stems	2.327±0.045	0.112	4.312	4.303		>4.303
	Leaves	1.940±0.046	0.114	4.286	4.303	<4.303	

Table. 5 Q test Rejection test of suspected values of different parts of plant of various concentration of lead after third week

Concentration (mg/L)	Plant parts	H_1 rejection ($t > t_v$)	Ranges of replicates (mg/L)			Difference between largest to nearest neighbor value	Difference between largest value and smallest value	Rejection Quotient at probability 95% (Q_{95})	$Q < Q_{95}$
50	Stems	>4.303	1.93	1.94	1.98	0.04	0.05	0.97	0.80
	Leaves	>4.303	1.04	1.06	1.09	0.03	0.05	0.97	0.60
100	Roots	>4.303	2.72	2.77	2.83	0.06	0.11	0.97	0.55
	Leaves	>4.303	1.69	1.74	1.76	0.02	0.07	0.97	0.29
150	Stems	>4.303	2.28	2.33	2.37	0.04	0.09	0.97	0.44

Table. 6 Student T tests statistical analysis of different parts of *Moringa oleifera* plant against different concentration of Pb after fourth week

Concentration (mg/L)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t_v)	H_1 rejection ($t < t_v$)	H_0 rejection ($t > t_v$)
0	Roots	0.033±0.006	0.014	4.141	4.303	<4.303	
	Stems	0.017±0.006	0.014	4.141	4.303	<4.303	
	Leaves	BDL	0.000				
50	Roots	2.837±0.074	0.183	4.286	4.303	<4.303	
	Stems	2.200±0.080	0.199	4.303	4.303		
	Leaves	1.767±0.071	0.176	4.299	4.303	<4.303	
100	Roots	2.960±0.036	0.090	4.309	4.303		>4.303
	Stems	2.347±0.100	0.249	4.310	4.303		>4.303
	Leaves	1.917±0.055	0.137	4.308	4.303		>4.303
150	Roots	3.227±0.083	0.207	4.317	4.303		>4.303
	Stems	2.903±0.060	0.150	4.323	4.303		>4.303
	Leaves	2.180±0.106	0.263	4.296	4.303	<4.303	

Table. 7 Q test Rejection test of suspected values of different parts of plant of various concentration of lead after fourth week

Concentration (mg/L)	Plant parts	H ₁ rejection (t > t _v)	Ranges of replicates (mg/L)			Difference between largest to nearest neighbor value	Difference between largest value and smallest value	Rejection Quotient at probability 95% (Q ₉₅)	Q < Q ₉₅
100	Roots	>4.303	2.92	2.97	2.99	0.02	0.07	0.97	0.29
	Leaves	>4.303	1.86	1.92	1.97	0.05	0.11	0.97	0.45
	Stems	>4.303	2.27	2.31	2.46	0.15	0.19	0.97	0.79
150	Roots	>4.303	3.16	3.20	3.32	0.12	0.16	0.97	0.75
	Stems	>4.303	2.84	2.91	2.96	0.05	0.12	0.97	0.42

Table. 8 Statistical analysis for magnesium after first week

Concentration (mg/L)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t _v)
0	Roots	1543±1.00	2.484	4.303	4.303
	Stems	1278±2.00	4.969	4.303	4.303
	Leaves	1156±2.00	4.969	4.303	4.303
50	Roots	3450±2.00	4.969	4.303	4.303
	Stems	2902±1.00	2.484	4.303	4.303
	Leaves	2679±1.00	2.484	4.303	4.303
100	Roots	3892±1.00	2.484	4.303	4.303
	Stems	3598±1.00	2.484	4.303	4.303
	Leaves	3420±1.00	2.484	4.303	4.303
150	Roots	3987±1.00	2.484	4.303	4.303
	Stems	3764±1.00	2.484	4.303	4.303
	Leaves	3684±2.00	4.969	4.303	4.303

Table. 9 Statistical analysis for magnesium after second week

Concentration (mg/L)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t _v)
0	Roots	1783±1.00	2.484	4.303	4.303
	Stem	1498±1.00	2.484	4.303	4.303
	Leaves	1276±4.00	9.938	4.303	4.303
50	Roots	3791±1.00	2.484	4.303	4.303
	Stem	3458±2.00	4.969	4.303	4.303
	Leaves	2899±1.00	2.484	4.303	4.303
100	Roots	4014±2.00	4.969	4.303	4.303
	Stem	3984±1.00	2.484	4.303	4.303
	Leaves	3874±1.00	2.484	4.303	4.303
150	Roots	4266±4.00	9.938	4.303	4.303
	Stem	4140±1.00	2.484	4.303	4.303
	Leaves	3974±1.00	2.484	4.303	4.303

Table 10. Student T tests statistical analysis of different parts of Moringa oleifera plant against different concentration of Mg after third week.

Concentration (mg/L)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t_v)	H_1 rejection ($t < t_v$)	H_0 rejection ($t > t_v$)
0	Roots	2086±3.00	7.453	4.301	4.303	<4.303	
	Stems	1849±3.00	7.453	4.301	4.303	<4.303	
	Leaves	1526±3.00	7.453	4.301	4.303	<4.303	
50	Roots	4110±1.00	2.484	4.303	4.303		
	Stems	4100±2.00	4.969	4.303	4.303		
	Leaves	3772±1.00	2.484	4.303	4.303		
100	Roots	4524±2.00	4.969	4.303	4.303		
	Stems	4230±2.00	4.969	4.303	4.303		
	Leaves	4137±5.13	12.749	4.309	4.303		>4.303
150	Roots	4872±4.00	9.938	4.303	4.303		
	Stems	4798±2.00	4.969	4.303	4.303		
	Leaves	4444±2.00	4.969	4.303	4.303		

Table. 11 Q test Rejection test of suspected value of plant part (leaves) at 100mg/L concentration of magnesium after third week

Concentration (mg/L)	Plant parts	H_1 rejection ($t > t_v$)	Ranges of replicates (mg/L)				Difference between largest to nearest neighbor value	Difference between largest value and smallest value	Rejection Quotient at probability 95% (Q_{95})	$Q < Q_{95}$
100	Leaves	>4.303	4133	4136	4143	7	10	0.97	0.70	

Table 12. Student T tests statistical analysis of different parts of Moringa oleifera plant against different concentration of magnesium after fourth week.

Concentration (mg/L)	Plant parts	Mean±SD	Accepted value	T test	Tabulated value (t_v)	H_1 rejection ($t < t_v$)	H_0 rejection ($t > t_v$)
0	Roots	2384±4.00	9.938	4.303	4.303		
	Stems	2088±1.00	2.484	4.303	4.303		
	Leaves	1730±2.00	4.969	4.303	4.303		
50	Roots	4487±1.00	2.484	4.303	4.303		
	Stems	4398±4.58	11.385	4.304	4.303		>4.303
	Leaves	4362±1.00	2.484	4.303	4.303		
100	Roots	5107±3.00	7.453	4.301	4.303	<4.303	
	Stems	4674±2.00	4.969	4.303	4.303		
	Leaves	4576±2.00	4.969	4.303	4.303		
150	Roots	5330±1.00	2.484	4.303	4.303		
	Stems	4984±1.00	2.484	4.303	4.303		
	Leaves	4895±1.00	2.484	4.303	4.303		

Table. 13 Q test Rejection test of suspected value of plant part (stems) at 50mg/L concentration of Mg.

Concentration (mg/L)	Plant parts	H ₁ rejection (t > t _v)	Ranges of replicates (mg/L)			Difference between largest to nearest neighbor value	Difference between largest value and smallest value	Rejection Quotient at probability 95% (Q ₉₅)	Q < Q ₉₅
50	Stems	>4.303	4393	4399	4402	3	6	0.97	0.50

50 mg/L Pb stems and leaves calculated Q values were 0.80 and 0.60 respectively. 100 mg/L Pb roots and leaves calculated Q values were 0.55 and 0.29 respectively. Similarly, 150 mg/L Pb stems calculated Q value was 0.44 less than tabulated value Q₉₅. 95% assured that suspected values are not rejected and error in the experiment may be due to indeterminate.(Ref: Table 5).

3.7 Absorbance of Lead (Pb) by different parts of Moringa oleifera plant against different concentration after fourth week

The highest amount accumulated in roots at 0 mg/L Pb, 50 mg/L Pb, 100 mg/L Pb and 150 mg/L Pb were 0.033 mg/L, 2.837 mg/L, 2.96 mg/L and 3.23 mg/L respectively. The maximum amount accumulated in stems at 0 mg/L Pb, 50 mg/L Pb, 100 mg/L Pb and 150 mg/L Pb were 0.017 mg/L, 2.20 mg/L, 2.35 mg/L and 2.90 mg/L respectively. Similarly, total absorbed concentration in 0 mg/L Pb, 50 mg/L Pb, 100 mg/L Pb and 150 mg/L Pb in leaves were BDL, 1.77 mg/L, 1.92 mg/L and 2.18 mg/L respectively.

Maximum absorbance of lead (Pb) in roots, stems and leaves when the concentration of solutions was 150 mg/L, 3.22 mg/L, 2.90 mg/L and 2.18 mg/L. The absorbance values increase in roots, stems, and leaves by increasing the concentration of solution but more in roots as compared to stems and more in stems as compared to leaves. It can be inferred that the maximum accumulation of lead was quantified in roots and minimum absorption was estimated in leaves. The descending order of Pb absorption is: Roots > Stems > Leaves.

3.8 Statistical Analysis

Student T tests statistical analysis of different parts of Moringa oleifera plant against different concentration of Pb after fourth week is shown in table 6.

Five cases were reported when $t > t_v$ that was 100 mg/L Pb Roots, Stems and leaves, calculated t values were 4.309, 4.310 and 4.308 respectively. 150 mg/L Pb Roots and Stems, calculated t values were 4.317 and 4.323. Both values were exceeded than tabulated value t_v (4.303). The suspected values can be tested by Q test to see suspected values are lie within the range at confidence limit 95% (Q₉₅) then suspected values

are not rejected and erroneous measurements may quite possible accidentally rather than determinate error. 100 mg/L Pb Roots, Stems and leaves, calculated Q values were 0.29, 0.45 and 0.79 respectively ($Q < Q_{95}$). Similarly, 150 mg/L Pb Roots and Stems calculated Q values were 0.75 and 0.42 respectively. 95% confidence that error in the experiments are not due to determinate and quite possible error may be occurred by chance or indeterminate error(Ref: Table 7).

3.9 Effect of Magnesium

3.9.1 Absorbance of Magnesium (Mg) by different parts of Moringa oleifera plant against different concentration after first week

The maximum concentration of magnesium accumulated in roots, Stems and leaves were 3987 mg/L, 3764 mg/L and 3684 mg/L respectively in 150 mg/L Mg. The estimated amount of Mg accumulated in roots, stems and leaves at 100 mg/L Mg were 3892 mg/L, 3598 mg/L and 3420 mg/L respectively. Similarly, at concentration of 50mg/L Mg the absorbed amount in Root, stems and leaves were 3450 mg/L, 2902 mg/L and 2679 mg/L respectively. In controlled group at 0 mg/L Mg in roots, stems and leaves the accumulations of Mg were 1543 mg/L, 1278 mg/L and 1156 mg/L.

The maximum magnesium absorption discerned in roots at 150 mg/L Mg was 3987mg/L, where maximum accumulation in Stems and leaves were 3764 mg/L and 3684mg/L. The accumulation of magnesium decreases as the strength of solution decrease.

3.10 Absorbance of Magnesium in Different Parts Of Plant After Second Week

Maximum absorbance at 150mg/L Mg in roots, leaves and stems were 4266mg/L, 3974mg/L and 4140mg/L respectively. At 100mg/L the absorbance values in roots, leaves and stems were 4014mg/L, 3874mg/L and 3984mg/L respectively. Similarly at concentration of 50mg/L Mg the absorbance values in roots, leaves and stems were 3791mg/L, 2899mg/L and 3458mg/L respectively. in controlled group at 0mg/L

concentration of Mg the absorbance values in roots, leaves and stems were 1783mg/L, 1276mg/L and 1498mg/L respectively (Table 8)

Maximum absorbance of magnesium (Mg) in roots, stems and leaves were 4266mg/L, 4140mg/L and 3974mg/L respectively when the concentration of solution is 150 mg/L. The absorbance values were decreases as the concentration of solution decrease from 150mg/L to 0mg/L Mg. T values were equal to tabulated value t_v (4.303) So all the values were acceptable (Ref: Table 9).

3.11 Absorbance of Magnesium in different parts of plant after third week

Concentration of magnesium accumulated in roots at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg were 2086mg/L, 4110mg/L, 4524mg/L and 4872mg/L respectively. Estimated amount of magnesium accumulated in leaves at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg were 1526mg/L, 3772mg/L, 4137mg/L and 4444mg/L respectively. Similarly, in absorbance in stems at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg were 1849mg/L, 4100mg/L, 4230mg/L and 4798mg/L respectively.

In controlled group the absorbance of magnesium at concentration of 0mg/L Mg in roots, stems and leaves were 2086mg/L, 1849mg/L and 1526mg/L respectively. Absorbance of magnesium at 50mg/L Mg solution in roots, stems and leaves were 4110 mg/L, 4100 mg/L and 3772 mg/L respectively. When the concentration of solution was 100mg/L Mg then absorbance values in roots, stems and leaves were 4524mg/L, 4230mg/L and 4136mg/L respectively. Similarly, when concentration of solution was 150mg/L Mg accumulation of Mg in roots, stems and leaves were 4872mg/L, 4798mg/L and 4444mg/L respectively.

3.11.1 Statistical Analysis

Student T tests statistical analysis of different parts of Moringa oleifera plant against different concentration of Mg after third week is shown in table 10.

One case was noted when t value exceeded from tabulated value t_v (4.303). In 100 mg/L Mg leaves, t value was calculated 4.309 (Ref: Table 10).

100 mg/L Mg leaves calculated Q value was 0.70 less than tabulated value Q_{95} . 95% assured that suspected values are not rejected and error in the experiment may be due to indeterminate (Ref: Table 11).

3.12 Absorbance of Magnesium in different parts of plant after fourth week

As the concentration of Mg in solutions increases the absorbance of magnesium in plants parts also increases which is more beneficial for plant height. After the fourth week the highest accumulated magnesium in roots at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg were 2384mg/L, 4487mg/L, 5107mg/L and 5330mg/L respectively. Accumulated magnesium in leaves at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg were 1730mg/L, 4362mg/L, 4576mg/L and 4895mg/L respectively. Similarly, in stems at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg were 2088mg/L, 4398mg/L, 4674mg/L and 4984mg/L respectively.

In 0 mg/L Mg, the estimated absorption of Mg in roots, stems and leaves were 2384 mg/L, 2088 mg/L and 1730 mg/L respectively. In 50 mg/L Mg, the estimated absorption of Mg in roots, stems and leaves were 4487 mg/L, 4398 mg/L and 4362 mg/L respectively. In 100 mg/L Mg, the estimated absorption of Mg in roots, stems and leaves were 5107 mg/L, 4674 mg/L and 4576 mg/L respectively. In 150 mg/L Mg, the estimated absorption of Mg in roots, stems and leaves were 5330 mg/L, 4984 mg/L and 4895 mg/L respectively.

3.12.1 Statistical analysis

Student T tests statistical analysis of different parts of Moringa oleifera plant against different concentration of magnesium after fourth week is shown in table 12.

One case was reported when $t > t_v$ at 50mg/L Mg stems, calculated t value was 4.304 (Ref: Table 12).

Q value calculated at 50mg/L Mg in stems was 0.50 that was less than tabulated value ($Q < Q_{95}$). 95% probability replicate measurements are not outlier of the range and 95% assured that erroneous measurements may be occurred accidentally rather than determinate error in the experiment. So, results are not rejected (Ref: Table 13).

4. Comparative Relation Between Absorption of Lead In Roots Leaves & Stems.

Graph 1. Comparative relation between absorption of Lead in Roots leaves & stems after 1st week

Graph 2. Comparative relation between absorption of Lead in Roots leaves & stems after 2nd week

Graph 3. Comparative relation between absorption of Lead in Roots leaves & stems after 3rd week

Graph 4. Comparative relation between absorption of Lead in Roots leaves & stems after 4th week

Comparative relationship between absorption of lead in roots, leaves and Stems at different concentration of lead can be seen. It can be inferred that the maximum accumulation of lead was quantified in roots and minimum absorption was estimated in leaves. The descending order of Pb absorption is: Roots > Stems > Leaves.

Graph 5. Comparative relation between absorption of Magnesium in Roots leaves & stems after 1st week

The comparative relationship between absorbance and plant's parts at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg. The absorbance values of magnesium were highest in roots as compared to stems and the absorbance values of magnesium were more in stems as compared to leaves. Descending order of magnesium present in different parts of plants was: roots > stems > leaves.

Graph 6. Comparative relation between absorption of Magnesium in Roots leaves & stems after 2nd week

Graph 7. Comparative relation between absorption of Magnesium in Roots leaves & stems after 3rd week

Graph 8. Comparative relation between absorption of Magnesium in Roots leaves & stems after 4th week

Both metals lead and magnesium affect differently at plants growth. Absorbance of lead increases the growth of plants by increasing the concentration of lead in solution but by increasing the concentration of lead in solution color of leaves become yellowish. On the other hand, by increasing the concentration of magnesium in solution growth of plants increases more than lead solution and color of leaves were green. So, we can say that magnesium is more beneficial for the plant growth.

Analysis shows that magnesium was also present in controlled groups of *Moringa oleifera* plants. As the concentration of magnesium in solutions increases amount of magnesium in plants parts increases. But lead was not present in controlled group as the concentration of lead in solution increases the concentration of lead was become detectable via AAS.

The accumulation of heavy metals lead and magnesium in different parts were in descending order Roots > Stems > Leaves. Plant height for lead at 0 mg/L Pb, 50 mg/L Pb, 100 mg/L Pb and 150 mg/L Pb was 68.23cm, 70.21cm, 65.38cm and 61.08cm respectively.

Figure 1: Effect of Pb on Moringa plant after four weeks

Figure 2: Effect of Mg on Moringa plant after four weeks

Plant height for Magnesium at 0mg/L Mg, 50mg/L Mg, 100mg/L Mg and 150mg/L Mg was 68.23cm, 74.03cm, 79.08cm and 82.03cm respectively.

5. Conclusion

Soil contaminated with lead was dangerous to plant growth when the concentration of lead solution become 100mg/L Pb and 150mg/L Pb. At 50mg/L Pb Moringa oleifera plant not more effective. In this way medicinal use of Moringa plant also affected. On the other hand, the growth of Moringa oleifera plants increased as the concentration of magnesium in solution increases. Magnesium as a macronutrient has high tolerance index and Lead is toxic metal and transported to all parts of plants.

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7. References

1. S.H. Maja, M.K. Habauka, M.N. Fiona, Structure of flocs of latex particles formed by addition of protein from Moringa seeds, Colloids and Surfaces Physicochemical and Engineering Aspects, 460–467, 2013.
2. M.E. Abalaka, S.Y. Daniyan, S.B. Oyeleke, S.O. Adeyemo, The antibacterial evaluation of Moringa oleifera leaf extracts on selected bacterial pathogens, Food sciences and human wellness, (2), 1–4, 2012.
3. Akunna, G.G. Ogunmodede, O.S. Saalu, C.L. Ogunlade, B. Bello and E.O. Salawu, Ameliorative effect of Moringa oleifera leaf extracts on chromium-induced testicular toxicity in rat testes, World Journal Life Science. (2), 20-26, 2012.
4. T.A. Ternes, Occurrence of drugs In Germans ewage treatment plants and rivers dedicated to Professor Dr .Klaus Habereron theoccasion of his 70th birthday, Water Research journal of chemistry, (32), 3245–3260, 2012.
5. R. Paliwal, V. Sharma and Pracheta. A Review on Moringa oleifera, A Multipurpose Tree with High Economic and Commercial Importance, Asian Journal of Biotechnology, (3)4, 317-328, 2011.
6. R.Paliwal, V.Sharma, P. Sharma, Phytochemical analysis and evaluation of antioxidant activities of hydroethanolic extract of Moringa oleifera Lam, International journal of molecular sciences, (4), 554–557, 2011.
7. K.A. Yongabi, D.M Lewis, P.L. Harris, Indigenous plant based coagulants/disinfectants and sand filter media for surface water treatment in Bamenda, Cameroon, African Journal of Biotechnology, (10), 8625–8629.
8. T.S. Anjorin, P. Ikokoh, S. Okolo, Mineral composition of Moringa oleifera leaves, pods and seeds from two regions in Abuja, Nigeria, African journal of environmental science and technology, (12), 431–434, 2010.
9. J.L. Rockwood, B.G. Anderson, D.A. Casmatta, potential uses of Moringa oleifera and an examination of antibiotic efficacy conferred by Moringa oleifera seed and leaf extract using crude extraction techniques available to underserved indigenous populations, International journal phytotherapy, (3), 61-71, 2013.
10. A. Leone, A.Spada, A. attezzati, A. Schiraldi, J. Aristil, S. Bertoli, Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves, An overview of international journal of molecular sciences, (16), 12791–12835, 2015.
11. N.A. Kumar and L Pari. Antioxidant action of Moringa oleifera Lam. Against antitubercular drugs induced lipid peroxidation in rats, Journal of Medicinal Food, (6)3, 255-259, 2013.
12. A. Arzani, H. Zeinali, K. Razmjo, Iron and magnesium concentrations of mint accession, Asian pacific journal of tropical biomedicine, (45), 323-329, 2017.
13. E.N Ali, Removal of Copper from Water System using Moringa oleifera Press Cake, International Conference on Agricultural, Civil and Environmental Engineering, (16), 15-18, 2016.
14. I.N. Abdullahi, K. Ochi, A.B.Gwaram Plant population and fertilizer application effects on biomass productivity of Moringa oleifera in north central zone of Nigeria, Peak Journal of Agricultural Science, (1), 94-100, 2013.
15. M. S. Rakesh Sharma and N. S. Raju, Correlation of heavy metal contamination with soil properties of industrial areas of Mysore, Karnataka, India by cluster analysis, International Research Journal of Environment Sciences, (2), 22–27, 2013.