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Use of phytoremidation technique in reclamation of soils contaminated with some heavy metals (C0, Cu, Pb) by cultivating Canna generalis plant

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Abstract---A pot experiment was carried out in the wooden canopy of Musayyib Technical College / Department of Soil and Water Techniques located in Musayyib project using soil with a loam texture brought from Musayyib project field from surface layer (0-30 cm), to study the bioremediation of soil contaminated with some heavy metals (Co,Cu,Pb) using Canna generalis plant, to evaluate efficiency of this plant in bioremediation process of contaminated soil , accumulation of Lead, Copper and Cobalt in soil accordance with international standards for soil pollution. Young Canna generalis plant seedlings (one week old) were planted at a rate of two plants in 25 kg plastic pots in winter on 14/11/2021 for a period of three months, they were then reduced to one plant. Factorial experiment was carried out according to a complete randomized design (CRD) using Canna generalis plant, five different levels of heavy metals (CO, Cu, Pb) and three replications, bringing number of experimental units to 45 experimental units .Tretments are To, T1, T2, T3, and T4, including adding elements CO, Cu, and Pb separately at levels 0.0, 10, 20, 40, 60 mg kg^{-1}. Added macronutrient fertilizer (NPK) according to heavenly recommendation, plants were irrigated with tap water after 35% of prepared water was exhausted, soil samples were taken before and after end of experiment to estimate available and total concentrations of heavy metals. Soil environmental pollution standards were

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calculated, which included contamination factor (CF), pollution load index (PLI) and geological accumulation index (Igeo). Results showed, Increasing availabile concentration of Lead, Copper and Cobalt in soil at end of experiment, increase was direct with increase in levels of adding heavy metals, but it did not exceed the limits allowed by Food and Agriculture Organization of International and World Health. High-very high pollution in soil planted with Canna generalis plant in relation to pollution factor (CF) of Co, Cu, and Pb, and a direct relationship was found between the added quantities of metals and degree of contamination factor, there was highest degree of soil pollution in relation to pollution load index (PLI), which shows occurrence of corruption and deterioration of soil quality. Moderate pollution in relation to evidence of geological accumulation (Igeo) when adding Lead, and moderate - severe pollution in relation to evidence when adding Copper and Cobalt, in a direct relationship with added levels of heavy metals in soil.

***Keywords*---**phytoremidation technique, soils contaminated, heavy metals, Canna generalis plant.

Introduction

Technological advances and increasing population growth have increased the demand for consumer products and energy, which has led to an increase in construction of chemical Factories, nuclear power stations, and oil refineries. These facilities began dumping their waste loaded with various types of heavy metals, hazardous, toxic and radioactive materials into environment with all its components. air, water and soil , spread of polluted sites and accumulation of toxic pollutants from heavy elements such as Cadmium (Cd), Zinc (Zn), Lead (Pb), Copper (Cu), Cr, Mercury (Hg), Nickel (Ni), Cobalt (Co), radionuclides and organic pollutants in soil and water in large parts of world (Benni, 2014). What increases danger of these pollutants is that most of them are persistent pollutants capable of accumulating and biomagnifying in organism's body. Soil is polluted when it contains high concentrations of polluting substances that cause danger to humans, animals and plants (Al-Jubouri, 2016).

Soil represents final downstream of materials that are released to environment from various human activities, whether they are organic or mineral pollutants, which accumulate in soil due to its physicochemical properties, causing serious problems for environment in general (Horta et al., 2015). Idea of phytoremediation lies in use of plants with a high ability to absorb and accumulate heavy metals in their tissues without negatively affecting their growth or physiological behavior, as this technique is considered among promising solutions in treating problems of contaminated soils (Raskin et al., 1997). Goel et al. (2009) defined biological treatment or phytoremedation as sum of processes carried out by plants to absorb toxic and heavy elements from soil and water , Mudgel et,al (2010) called (Phytoremedation) green technology, which is method by which pollutant concentrations are reduced in soil through their absorption by cultivated plants, and it is an excellent way to remove pollutants and clean them from soil.

Biological treatment is a method of low cost and high efficiency compared to use of other chemical methods. In this process, plants are used that accumulate heavy elements and have a high ability to accumulate within plant tissues (Wendy, 2005; Mahmood, 2010; Karami and Shamsuddin, 2010). Phytoremediation helps improve contaminated soil and water by extracting polluted heavy metals, which is called phytoremediation, and fixing them in their plant bodies. Phytoremediation relies on many processes, and is highly environmentally friendly, cost-effective, and economical (Kumar Verma et al., 2022). Plants capable of treating contaminated soils are characterized by their ability to adapt under conditions of high levels of heavy metals, they are tolerant plants that are used as indicators and highly tolerant plants (Sarma, 2011 and Kramer, 2010).

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Phytoremediation is a modern technology that enables higher plants to clean polluted environment. Mechanisms by which plants promote removal of pollutants are varied, including uptake and concentration, conversion of pollutants, stabilization, and rhizosphere degradation, as plants promote growth of underground bacteria in root zone that in turn break down pollutants. (Abou Seeda et al., 2020). Scientists have discovered amazing abilities of green plants and trees, represented in their great potential in combating persistent pollutants, radioactive pollutants and heavy metals (Azita and sied, 2008). Canna generalis plant (Canna Indica) or Indian canna is a plant species of genus Canna, which is a perennial evergreen ornamental plant,fast-growing, may reach a meter or more in length, resembles a Canna tree, is characterized by large-sized leaves, and attractively colored flowers. Canna blooms in most days of year except for winter. It is considered a moisture-loving plant, it needs abundant watering, and direct sunlight. (Sultan and others, 1992). Research aims to evaluate efficiency of Canna generalis plant in reclaiming soils contaminated with some heavy metals (Co,Cu,Pb) and assessing soil pollution with these elements according to international soil pollution standards.

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Materials and Methods

An experiment pots with a capacity of 25 kg was carried out in wooden canopy of Technical College / Al-Musayyib / Department of Soil and Water Techniques located in Al-Musayyib project. Experiment was conducted in winter on November 14, 2021. Soil was brought from Musayyab project field to site of experiment with a loam texture. Soil was taken from surface layer at a depth of 0-30 cm . Initial physical and chemical laboratory analysis were carried out before cultivation according to methods mentioned in Black(1965) ,Page et al. (1982), Bashour and Al-Sayegh (2007) (Table 1), 20 kg of soil were placed in each pot. Heavy elements were added to soil as sulfurs for each element.

Table 1. Some physical and chemical properties of studied soil

Characteristics	Units	Value
ECe	$dS.m^{-1}$	3.08
pH	---------	7.65
Organic Matter	$g.Kg^{-1}$	6.30
$CaCO_3$	$g.Kg^{-1}$	272.50

CEC	Cmole + Kg^{-1}	18.70
Available Pb	$mgKg^{-1}$	0.04
Available Cu	$mgKg^{-1}$	0.17
Available Co	$mgKg^{-1}$	1.82
Total Pb	$mgKg^{-1}$	6.90
Total Cu	$mgKg^{-1}$	4.73
Total Co	$mgKg^{-1}$	4.95
Sand	$g.Kg^{-1}$	489.50
Silt	$g.Kg^{-1}$	376.20
Clay	$g.Kg^{-1}$	134.3
Soil Texture	Loam	

Heavy elements were added to soil in form of sulfurs for each element. Added quantities were calculated according to concentrations used (0, 10, 20, 40,60 mg kg^{-1} , symbols T0, T1, T2, T3, T4) were added to soil in form of solutions with field capacity moisture, mixed with soil before planting and left for a week for equilibrium while maintaining moisture content of soil by gravimetric method, Then seedlings of Canna generalis plant were planted at age of one week at a rate of two plants per pot, and after a week it was reduced to one plant.

Chemical fertilizers of NPK elements were added to soil according to fertilizer recommendation for each plant, and irrigation was carried out with valid river water according to weight method and continued irrigation after depletion of 35% of available water. Completely randomized design (CRD) was used using five different levels of heavy metals (Co, Cu, Pb) with three replications, number of experimental units is 45 experimental units. At end of experiment, soil samples were taken and concentrations of available and total heavy metals (Cu, Pb ,Co), were estimated.

Indicators of environmental pollution of soil (Hakanson,1980)
Contamination factor (CF)

CF = Ci / Si
Since:
CF: pollution factor
) Ci: concentration of element in contaminated sample ($mgkg^{-1}$
Si: concentration of element in reference sample (mg kg^{-1})

Table 2. Contamination factor index values

classification	Valu CF
Low Contamination	≤1
Moderate Contamination	>1>3
Considerable Contamination	>3>6
Very high Contamination	>6

Pollution Load Index (PLI) (Hakanson,1980)

$^{n/}$ PLI = (CF1 x CF2 x CF3 x…….. x CFn)^{1}

Since:
CF1 : Pollution factor for first element.
CF2 : Pollution factor for second element.
CF3 : Pollution factor for third element.
n : Number of contaminated items.

Table 3. Pollution load index values

Value PLI	Classification
PLI < 1	Indicate uncontaminated site
1 = PLI	Pollutant ara present only at baseline levels
PLI >1	Indicate deterioration of site quality

Igeo Geoaccumulation Index

C sample
[----------------] Igeo = log 2
1.5 C control
Since:
C sample: concentration of e pollutant element in soil (mg-kg^{-1})
C control: concentration of pollutant in comparison model (mg kg^{-1})
1.5 : coefficient of geological accumulation (due to influence of genetic rock factor).

Table 4. Geological accumulation index values

Igeo value	Igeo class	Designation of soil quality
<0	0	Unpolluted
>0 >1	1	unpolluted to moderately polluted
>1 >2	2	moderately polluted
>2 >3	3	Moderately to strongly polluted
>3 >4	4	strongly polluted
>4 >5	5	strongly polluted to very strongly polluted
>5	6	very strongly polluted

Results and Discussion

Available heavy metals in soil after end of experiment

Results of Table 7 show available concentrations of elements pb, Cu and Co in soil after end of experiment. it showed that there were significant differences between average concentrations of available Lead, Copper and Cobalt in soil at different levels of adding elements ,it was (0.03, 0.09, 0.27, 0.55, 1.09) , (0.17, 0.32, 0.67, 1.19, 2.65) and (1.08, 1.21, 1.87, 2.71, 3.61) mg kg^{-1} for above elements in succession for treatments T0, T1, T2, T3, T4 sequentially.

Table 5. Concentrations of available Lead, Copper and Cobalt (mg kg^{-1}) in soil after end of experiment

Tretments	Co	Cu	Pb
T0	1.08	0.17	0.03
T1	1.21	0.32	0.09
T2	1.87	0.67	0.27
T3	2.71	1.19	0.55
T4	3.61	2.65	1.09
LSD*0.05*	1.435	0.941	0.243
Mean	2.10	1.0	0.41
LSD*0.05*	0.419		

Increase in concentrations of elements for different treatments is due to increase in levels of addition of these elements to soil. It is noted in table that finished Cobalt concentrations were superior to Copper, which in turn outperformed Lead, as highest concentration of Cobalt reached 3.61 mg kg^{-1} at T4, while it reached 2.65 for Copper, which in turn is higher than Lead, which is 1.09 mg kg^{-1} soil. This may be due to increase in Lead deposition in calcareous soils in form of hydroxides and carbonates, in addition to exposure of element to adsorption and stabilization reactions by silicate minerals and carbonate minerals due to relative high degree of soil pH compared with Copper and Cobalt, this is consistent with Al-Gawam and Al-Rabiei (2016), who showed that high degree of soil pH leads to deposition of heavy metals in form of hydroxides, and is consistent with Al-Halfi (2010), which showed that soils with a high content of carbonate minerals have a great ability to adsorb or precipitate heavy metals in soil as a carbonates. Increase in concentrations of available Lead in soil for different treatments did not exceed critical limits allowed by Food and World Health Organization (WHO / FAO, 2007) amount of 100 mg Pb kg^{-1} soil, this is consistent with Odeh (2018) and Abdul Latif (2016), who established that increase in concentrations of available Lead in soil after planting is directly proportional to increase in added concentrations at each treatment. It is also noted that concentrations of available Copper and Cobalt in soil did not exceed limits allowed by Food and Health Organization (WHO/FAO, 2007) of 100 mg kg^{-1} soil and 11 mg kg^{-1} soil for two elements, respectively. Highest value of prepared Copper in T4 treatment was 2.65 mg kg^{-1} soil and Cobalt also for same treatment was 3.61 mg kg^{-1} soil, while average concentrations of Copper were 0.94 and Cobalt 1.43 mg kg^{-1} soil.

Total heavy metals in soil after end of experiment

Results of Table 6 show significant increase in concentrations of total heavy metals in soil (mg kg^{-1} soil) after end of experiment, concentrations of Lead increased significantly for different treatments and reached 5.0 , 10.5, 11.73, 17.41, 26.12 , for Copper 0.40, 5.13, 2.47, 15.01 and 14.00 and for cobalt 0.51, 4.02, 1.74, 9.21, 7.18 mg kg^{-1} for treatments T0, T1, T2, T3, T4 Successively, it is due to increase in added

Table 6. Total concentrations of Lead, Copper and Cobalt (mg kg^{-1}) in soil after end of experiment

Tretments	Co	Cu	Pb
T0	0.51	0.40	5.00
T1	4.02	5.13	10.5
T2	1.74	2.47	11.73
T3	9.21	15.01	17.41
T4	7.18	14.00	26.12
LSD*0.05*	4.847	4.508	3.558
Mean	4.532	7.402	14.152
LSD*0.05*	3.575		

levels of elements and their accumulation in soil as a result of their sedimentation or adsorption reactions, especially in alkaline soils, this is consistent with Al-Kawam and Al-Rubaie (2016).

In addition to influence of soil texture on this, Nordberg et al. (2015) indicated that total Cu content is closely correlated with soil texture. Silty clay soils contain a high level of total Cu. It appears in table that total concentrations of Cobalt increased compared to Copper, which had a higher cycle than Lead, as it reached 4.53, 4.50, 3.55 mg kg^{-1} for elements in succession, this is consistent with the concentrations of these available elements in soil, which were in same order (Table 1).

Increases in total concentration of Lead in soil after end of experiment were within internationally permissible limits and did not exceed them, which amounted to 100 mg pb kg^{-1} soil according to Food and Health Organization (WHO/FAO, 2007), It is also within average concentration of Lead in soil globally (17-65 mg kg^{-1} according to Pendias-kabata, 2011). Copper concentrations did not exceed critical limits allowed internationally according to (WHO/FAO, 2007), which is 100 mg kg^{-1}, which is located within range of total Cu concentration in calcareous soil (10-70 mg kg^{-1}) it is below critical limits range, which ranges between 60-150 mg Cu kg^{-1} soil, according to Pendias- kabata, 2011). Also, total Cobalt concentrations in soil did not exceed critical limits of 20-50 mg Co kg^{-1} soil, according to Pendias- kabata. , 2011).

Soil Environmental Pollution Standards
Contamination factor (CF)

CF pollution factor is used to assess soil pollution for a single element, on which most other indicators of pollution depend, and degree of pollution includes sum of pollution factors of studied elements and is used to measure total degree of soil pollution (Asrari, 2014,).

Contamination factor for Lead (CF-pb)

Results of Table 7 show that there are differences in values of contamination factor of Lead for soil after end of experiment. Highest values for treatments T3 and T4 were 6.378 and 9.032, which are within very high contamination range because values greater than 6($CF \geq 6$) followed by T2 which falls within range of high contamination $3 \leq CF < 6$ then T1 which falls within range of moderate contamination $1 \leq CF < 3$ and finally comparison treatment T0 which falls within range Low contamination $CF < 1$.

Table 7. Contamination factor values of Lead in soil for different treatments after end of experiment

Tretments	CF Value	Classification
T0	0.729	Low contamination
T1	1.521	Moderate contamination
T2	3.016	Considerable contamination
T3	6.378	Very high contamination
T4	9.032	Very high contamination

Increase in pollution factor for different treatments is due to increase in concentration of Lead element in soil by increasing levels of addition ,this is consistent with Chen (2016) and Odah (2018), who showed that soil contamination with heavy metals leads to higher levels of availabile form and finally leads to higher values of pollution factor compared to unpolluted soils.

Contamination factor for Copper (CF-Cu)

Results of Table 8 showed that there were differences in values of soil contamination factor with Copper, at end of experiment, highest values were in two treatments T4 and T3 which amounted to 8.668 and 12,401 that fall within range of extreme pollution , then T2, which amounted to 4.613 which falls within range of high pollution, followed by T1, which falls within range of moderate pollution, which amounted to 1.750, and finally comparison treatment T0, which was within range of low pollution, with a value of 0.729.

Table 8. Contamination factor values of Copper in soil for different treatments after end of experiment

Tretments	CF Value	Classification
T0	0.729	Low contamination
T1	1.750	Moderate contamination
T2	4.613	Considerable contamination
T3	8.668	Very high contamination
T4	12.401	Very high contamination

Increase in values of soil contamination factor with Copper is attributed to escalating additions of Copper to soil, which led to an increase in availability of it in soil, in addition to increase in total concentrations of Copper in soil, which in turn led to an increase in values of Copper contamination factor, especially T2,T3 and T4. These results are consistent with Al-Asadi et.al., (2011), who showed that increase in concentration of heavy metals in soil increases availability of elements and their total quantity in soil, which leads to an increase in values of pollution factor.

Cobalt Contamination Factor (CF-Co)

Results of Table 9 indicate that there are differences between different treatments in values of soil pollution factor in Cobalt. Highest values were in two treatments T3 and T4, which fall within range of very high pollution, as values reached 9.044 ,13.125, followed by T2, which falls within range of high pollution, values reached 5.319, and finally, T1, T0 which fall within range of moderate pollution, with a value of2.687, 1.210, respectively , this is attributed to increased additions of Cobalt to soil, especially to the treatments T4, T3, T2, which led to an increase in available and total Cobalt concentrations in soil (Tables 7and 8), which positively affected values of Cobalt pollution factor in soil.

Table 9. Contamination factor values of Cobalt in soil for different treatments after end of experiment

Tretments	CF Value	Classification
T0	1.210	Moderate contamination
T1	2.687	Moderate contamination
T2	5.319	Considerable contamination
T3	9.044	Very high contamination
T4	13.125	Very high contamination

These results are consistent with Kebir and Bouhadjera(2011), who showed that increase in concentration of available heavy metals in soil increases its total concentration, which leads to an increase in values of pollution factor for these heavy metals.

Pollution load index (PLI)

Results of Table 10 indicated that all values of treatments used in study except comparison treatment (T0) for the soil planted with Canna generalis plant,at highest degree of soil contamination.

Table 10. Soil pollution load index values for e different treatments after end of experiment

Tretments	PLI Value	Classification
T0	0.643	Indicate uncontaminated site
T1	7.125	Indicate deterioration of site quality
T2	74.002	Indicate deterioration of site quality
T3	499.993	Indicate deterioration of site quality
T4	1470.076	Indicate deterioration of site quality

It is within range of soil quality deterioration (indicate deterioration of site quality) because PLI > 1, however, values of this indicator differed according to levels of addition for each treatment. Highest values were in treatment T4 and then in T3, T2, T1, reached 1470.076, 499.993, , 74.002, 7.125, while it reached in comparison treatment 0.643, which falls within range of best level because PLI values are less than one. Increase in pollution load index values is due to increased additions of heavy metals to soil, especially in treatments T2, T3, T4. These results are consistent with Odeh (2018), which shows that increasing levels of addition of heavy metals to soil, leads to an increase in availabile and total concentrations of these elements, which leads to an increase in the value of the pollution load index.

Geological Accumulation Index (Igeo)

This indicator was proposed by Muller (1969) , it is a quantitative measure used to determine mineral pollution of soil by comparing measured concentrations with local reference levels of those elements and determining extent of contribution of ground source to mineral pollution.

Geological accumulation index of Lead (Igeo-Pb.)

The results of Table 11 indicate that there are differences in values of index of geological accumulation of Lead index in soil for different treatments. Treatments T0, T1, T2 fell within non-polluted range to moderate pollution, as values reached 0.146, 0.305, 0.605 for above treatments in succession.

Table 11. Geological accumulation index (Igeo) for Lead element in soil for different treatments after end of experiment

Tretments	Igeo value	Classification
T0	0.146	Uncontaminated to moderately contaminated
T1	0.305	Uncontaminated to moderately contaminated
T2	0.605	Uncontaminated to moderately contaminated
T3	1.280	moderately contaminated
T4	1.812	moderately contaminated

low index values here may be attributed to low additions of Lead to soil and perhaps to its interaction with carbonate minerals in soil to form deposited Lead carbons, or adsorption of Lead ions on exchange surfaces in soil, especially that soil texture is a loam. Treatments T3 and T3, within moderate polluting range, where index value was,1.280, 1.812 respectively. High index values in these two treatments is due to addition of high levels of Lead to soil ,This is consistent with Odeh (2018), who showed that reason for high values of geological accumulation index of Lead in soil is due to high additions of Lead to soil.

Geological accumulation index of Copper (Igeo-Cu)

Results of Table 12 indicate different values of geological accumulation index of Copper in soil. Treatments T0, T1, T2 were similar in their occurrence within same range, unpolluted to moderate pollution, values reached0.146 , 0.351, 0.925 for above treatments in succession , all values are less than one (1> Igeo> 0).

Table 12. Geological accumulation index of Copper (Igeo-Cu) in soil for different treatments after end of experiment

Tretments	Igeo value	Classification
T0	0.146	Uncontaminated to moderately contaminated
T1	0.351	Uncontaminated to moderately contaminated
T2	0.925	Uncontaminated to moderately contaminated
T3	1.739	moderately contaminated
T4	2.488	Moderate to strongly contaminated

This may be attributed to low levels of Copper added to soil in treatments T1 and T2 and that the plant absorbed Copper as it is one of nutrients that plant needs, therefore, no geological accumulation of Copper appeared in these treatments, this is consistent with Mafuai et al. (2015), who showed that concentration of micro-nutrients added to soil decreases due to plant's absorption of them, and thus values of geological accumulation index in soil decrease. Values of index for treatment T3, increased to be greater than one, reaching 1.739, which falls within range of moderate pollution. Values increased more in treatment T4, to fall within range of moderate to severe pollution, which amounted to 2.488. High values of geological accumulation index of Copper in treatments T4 and T3 may be attributed to high additions of Copper to soil. These results are consistent with Bempah et al. (2011), who explained that increase in soil pollution with heavy metals leads to an increase in value of soil pollution indicators.

Geological accumulation index of Cobalt (Igeo-Co)

Results of Table 13 show variation of different treatments in values of geological accumulation index of Cobalt in soil after end of experiment, treatments T0 and T1 were within same range, unpolluted to moderate pollution, because their value was less than one ($1 < Igeo > 0$) and this may be attributed to small amount of Cobalt added at treatment T1, which was absorbed by plant because Cobalt is one of micro-nutrients needed by plant (Awdah, 2018).

Table 13. Geological accumulation index (Igeo.) of Cobalt in soil for different treatments after end of experiment

Tretments	Igeo value	Classification
T0	0.242	Uncontaminated to moderately contaminated
T1	0.539	Uncontaminated to moderately contaminated
T2	1.067	moderately contaminated
T3	1.814	moderately contaminated
T4	2.633	Moderate to strongly contaminated

Index values slightly increased to reach in T2 and T3 to extent of moderate pollution, where values amounted to 1,067 and 1,814 respectively. ($1<Igeo<2$), value increased in T4 to 2.633, which is within moderate to severely polluted range ($2<Igeo<3$). Increase in values in soil at treatments T2, T3 and T4 is attributed to increased additions of Cobalt to soil. These results are consistent with Al-Asadi et. Al., (2011), who indicated that increasing levels of heavy metals addition to soil leads to an increase in their concentration in soil, an increase in soil pollution, and an increase in values of environmental pollution indicators.

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