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Phytoremediation Capabilities of Sunflower (*Helianthus Annuus L.*) in Heavy Metal (As and Cd) Contaminated Soil

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Abstract

The study investigated the phytoremediation capabilities of oil seed plant (*Helianthus annus)*. The plant was grown on potted soils polluted with heavy metals (As and Cd) contaminant in a randomized complete block design (RCBD) and analyzed to evaluate its efficacy for the removal of As and Cd. Results obtained from the preliminary analysis of the soil physicochemical characteristics were within limits for plant optimal growth. There were variations in heavy metal concentration in the different parts (roots, stems, leaves and seeds) of test plants and significant difference ($P<0.05$) between plant part following a decreasing order of concentration. (300>200>100>0) mg/kg, leaf – Cd content was higher than As content, in the following order: Cd > As and in a decreasing order as per plant parts as follows: leaf > stem > root > seeds. The application of metal concentration in soil increases as metal-ion, uptake in plant increases across the treatments as follows: 300mg/kg > 200mg/kg > 100mg/kg > 0mg/kg. Result indicated that heavy metals concentration in whole and plant parts, exception of the seeds were above the WHO/FAO recommended limits. The general trend of heavy metal accumulation in plants parts of *H.annus* in terms of Bio concentration factors indicated that *H. annuus* is an hyper-accumulator of heavy metals having exceeded BCF bench mark of 1 > 10. and is therefore classified as high efficiency plants for metal translocation from root to shoot as the transfer factors were above TF value > 1. However, *the plant* showed symptoms of phytoxicity, especially when heavy metals were applied at high concentrations (300mg/kg) as evidence in stunted or growth retardation as well as yellowing of leaves and low yield .

Keywords: capabilities, contaminated soil, heavy metal, phytoremediation

INTRODUCTION

Pollution consists of different pollutants or constituent elements, pre-dominant among them are the heavy metals. However heavy metal refers to any metallic element where density is relatively high and is toxic or poisonous even at low concentration (Ibeanusi et al., 2014). They are often described as metals and metalloids having densities greater than 5g/cm (Bhatia, 2007). Some of which are useful in less concentration but can be harmful to the environment when they are in excess of the required quantity in any environmental medium (Ogwo et al., 2014). Potentially contaminated soils may occur at sites, particularly exposed to industrial waste or domestic waste, cultivated fields and agricultural plantation due to excessive use of pesticides or fields that has post-application of industrial sludge, tailings from legal or illegal mining of solid minerals, municipal wastes, chemical and radioactive waste deposition and hydrocarbon from crude oil spillage. These however may lead to contamination of both surface, underground water and land, thus becoming potential threats to humans and Livestocks (Jabeen et al., 2009; Shao et al., 2010). The fate and transport of these pollutants assumes different path ways but get accumulated in tissues of plants and animals in a process called bioaccumulation (Ubuoh et al., 2016). They can have acute or chronic effects on both plants and animals system and can also be transferred to the next generation. The primary concerns with heavy metals contamination is that of indestructibility by degradation and cannot be detoxified. Hence, this research seeks to explore the effectiveness of phytoremediation as a means of decontamination of polluted sites by heavy metals.

Materials and method

Determination of physico-chemical quality of soil sample

Various soil parameters such as soil texture, pH, Electrical Conductivity, organic carbon, water soluble sodium, potassium, calcium, exchangeable sodium, potassium and calcium, total nitrogen, water soluble phosphorus, chloride and sulphate, available phosphorus, cation exchange capacity was analyzed following standard laboratory procedures.

Determination of Heavy Metals in the Soil Samples

The instrument was calibrated using calibration blank and five series of the working standard solutions of each metal to be analysed. The digested samples were determined for the concentrations of heavy metals (Cd and As) using Flame Atomic Absorption Spectrophotometer (FAAS, Model: AA-329N, Shanghai, China).

Heavy metals contents of the samples soils were determined before and after planting following the method of Umeham and Okereke (2005).

Experimental Design

The study was carried out in a greenhouse condition, to determine the Phytoremediation Capabilities of Sunflower (*Helianthus annuus L.*) plant in heavy metal (As and Cd) contaminated soil.

The experiment was conducted in pots using Randomized Complete Block Design (RCBD) with three (3) replications. Four heavy metals (As and Cd) or their salts was applied to the pots containing pretested soils.

Experimental Treatments

After preliminary or initial analysis of soil parameters, 10kg of the pre-tested soil samples was transferred into 27plastic (10L) pots (36cm in diameter) with holes at the bottom for proper aeration. The plastic pots ¾ were filled with the soil samples. The pots were divided into 3 groups having 3 replications. Group 1-2 was contaminated artificially with two different heavy metal (As and Cd) or their salts of different concentrations (100mg/kg, 200mg/kg and 300mg/kg) of soils, while the third group was without addition of heavy metal (0 kg/mg - pure soils) for experimental control; each group was labeled accordingly.

Procedure for Determination of Heavy Metals Concentration in Different parts of the plants under study

Digestion of Plant Samples

Plant samples were oven dried for 48hrs at 30^0C. 1g each of the dried sample was digested with 15ml of concentrated nitric acid (HNO_3) overnight. Digested sample was heated at 25^0C until white fumes are observed and heavy metals concentration was determined by using Atomic Absorption spectrophotometer (AAS, Accusys 211 Buck Scientific, USA) at corresponding different wave length of heavy metals present. Data on concentration of heavy metals in different parts (roots, stem, leaves, flowers or fruits sets and seeds) of each plant was elicited following the method of Umeham and Okereke (2005).

Estimation of phytoremediation parameters

Phytoremediation parameters were calculated in terms of bioconcentration factors (BCF) and translocation or transfers factors (TF).

Result and Discussion

Determination of Physiochemical Quality of the soil

Table 1.1: Shows the Physicochemical Characteristics of the experimental soil, the percentage sand, silt and clay were mean values of 72.78 % ±2.0307, 10.11%±1.1846 and 17.13%±2.2923 respectively. The textural class was Loam with an average organic matter content of 3.5167 % of ±0.9943. The pH and Ec values of the studied soil were 5.1830±0.2082 and 0.1833ds/m ±0.0961 respectively. The soil had organic carbon content of 3.5167% ± 0.9943 and total nitrogen of 0.0900% ± 0.0265. Total available phosphorus was 39.58mg/kg ± 8.5771 with base saturation of 76.25% ± 10.2155. The EA and CEC were 1.48 cmol/kg ± 0.1600 and 6.8067C mol/kg ± 2.0243 respectively. The soil had relative amount of total As and Cd with mean concentration of 0.9593 mg/kg ± 0.0164 and 5.1418 mg/kg ± 2.2436 respectively. These Were below the WHO/FAO limit of heavy metals in soils except that of cadmium which was above the required limit in soils.

Table 1.1: Physicochemical Characteristics of the Experimental soil

Parameters	Units	Mean±SD
Sand	-	72.78±2.03
Silt	%	10.11±1.18
Clay	-	17.13±2.29
T-class	Loam soil	-
PH	H2O	5.18±0.21
Ec	ds/m	0.18±0.09
Organic carbon	%	2.03±0.57
Organic matter	%	3.52±0.99
Total Nitrogen	%	0.09±0.03
Av. Phosphorus	mg/kg	39.58±8.58
EA	Cmol/kg	1.48±0.16
ECEC	Cmol/kg	6.81±2.02
Base saturation	%	76.25±10.22
As	mg/kg	0.96±0.02
Cd	mg/kg	5.14± 0.24

Results are Means and S.D of Three Determinations.

Concentration level of Heavy metal (mg/kg) in Sunflower *(Helianthus annuus)*

Result presents result of heavy metals (As, Cd,) uptake at different levels of concentration (0,100,200,300) mg/kg in sunflower plant *(Helianthus annuus).* The result reveals metal (As) concentration level absorbed by the plant to be highest at 300 mg/kg for soil- Arsenic with mean value of 24.8967 ± 15.85555 and range from (1.06 – 43.48) followed by As 200 mg/kg of soil concentration having mean value of 16.4492 ± 10.22412 and ranged from (0.48 – 27.03), followed by mean value of 7.8017 ± 4.8907 at Arsenic concentration level of 100 mg/kg with the range of (0.27 – 14.07). The lowest mean concentration absorbed was plant grown on control soil at 0 mg/kg concentration level with mean value of 0.1288 ± 0.07808 with the range of (0 – 0.19). cadmium (Cd) absorption followed a similar trend with the highest mean value of 41.6983 ± 28.86207 but ranged from (2.112 – 81.9) and the lowest mean concentration value of 1.18 ± 0.79418 and ranged from (0 – 2.21) at 0 mg/kg of control soil for Cd in the following pattern 300 mg/kg > 200 mg/kg > 100 mg/kg> 0 mg/kg.

Concentration of Heavy metals in parts of sunflower *(Helianthus annuus)*

Data reveals levels in terms of concentration of heavy metals up -take in parts of sunflower plant. The result present mean values of Arsenic concentration in different parts of the plant mopped up by the plant from all levels of Arsenic contamination in the soils to be highest in leaf recording a mean value of 42.660± 0.78307 at 300mg/kg of soil – As, followed by stem with mean concentration value of 31.3300±1.08042 at 300mg/kg level of arsenic contamination in the soil. The least mean value was found in control seed at 0 mg/kg of 0.0000 ± 0.00000. The result also showed ranged of (41.885 – 43.435) for highest concentration (leaf) and least in seed ranged from (-0.775 – 0.775) from the result it is observed that Arsenic present a decreasing order of concentration in parts of sunflower as follows: Leaf > stem > root >seed , result shows levels of concentration of the cd- content in leaves to be highest with mean value of 80.4867 ± 1.54329 at 300 mg/kg and lowest in seed with mean value of 0.0000 ± 0.00000 at 0 mg/kg control-soil. Result shows a decreasing pattern of cadmium concentration in plant tissues of *Helianthus annuus*. As follows: leaf > stem > root > seed.

Evaluation of Phytoremediation potentials parameters in plant, sun flower plant *(Helianthus annuus*)

The ability of a plant to accumulate metals from contaminated soil has commonly been measured by phytoremediation parameters, calculated or evaluated in term of Bioconcentration factor (BCF), transfer or translation factor (TF) (Cui et al, 2004).

Bioconcentration factor of heavy metals in parts of *Helinathus annuus*

The bio-concentration factors of heavy metal concentration in parts of *Helianthus annuus* grown on different metal concentration levels (100, 200, 300) mg/kg is shown as follows respectively ; As 0.0801, 0.0859, 0.0811 (root); 0.1018, 0.1081, 0.1044 (stem); 0.0016,0.1325,0.1422 (leaf); 0.0029,0.0027,0.0039 (seed). Cd: 0.1528, 0.1547, 0.1361 (root), 0.2147, 0.2231, 0.2683 (stem); 0.2147, 0.2230, 0.2683(leaf); 0.0139, 0; 0106, 0.0080 (seed). The result further reveal that the BCF for*H. annuus* plant grown on soil without heavy metal contamination (0mg/kg) used as control was non-detectable. While the lowest detectable BCF were recorded in seeds as respectively follows: 0.0027, 0.0080, 0.0070 and 0.0014 for 100mg/kg soil-As, 300mg/kg soil-Cd.

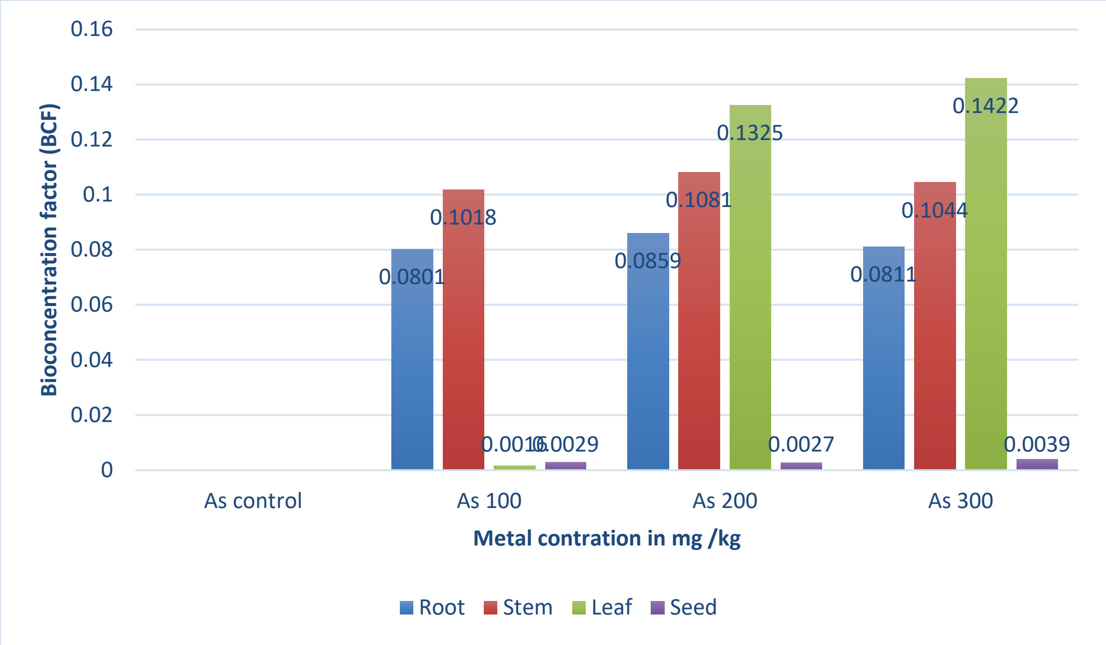

Figure 1.1: **Bioconcentration Factor (As) in *H.annuus***

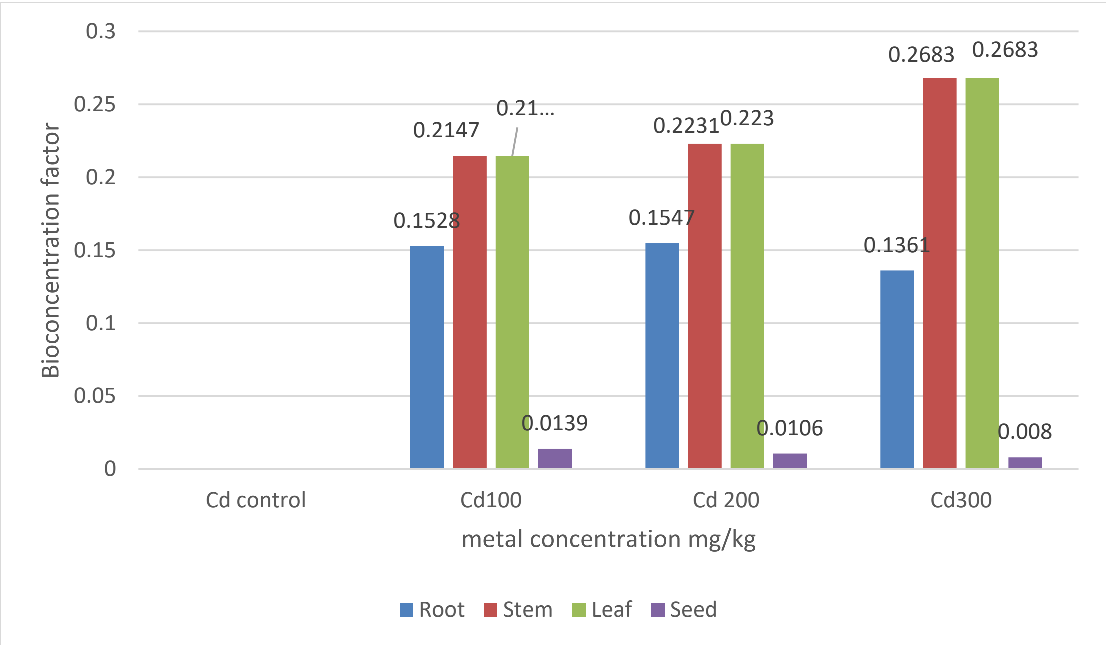

Figure 1.2: Bioconcentration Factor of Cd in *H. annuus*

Transfer factor in plant part of Helianthus annuus

Result reveals the ability of plant *(Helianthus annuus)* to translocate metal from contamination soils to its aerial parts of plant accordingly highest transfer value of 2.985was found in sunflower leaves grown on soil amended with cadmium (Cd) at 300 mg/kg .The transfer factor was lowest in seeds under control- soil without amendment with TF value of 0.000 and across seeds of *H. annuus* from other soils amended with different degree of heavy metal (As and Cd,) concentrations, with cadmium (Cd) having the least TF of 0.000, 0.071, 0.040 and 0.0386 for 0, 100, 200, 300mg/kg concentration of soil-cdrespectively.Cd was higher in leaves than stems and seeds of *H. annuus* are at all concentration levels with TF values of 0.921, 2.460, 2.219, 2.985 and 0.905, 2.556, 2.036, 2.705 respectively. While As was highest in stems than leaves and seeds with TF values of 0.933, 1.271, 1.261, 1.288 and 0.851, 1.576, 1.295, 1.423 at concentration levels of 0, 100, 200, 300 mg/kg respectively.Generally, the pattern of transfer from root to stem, leaves and seed, were as follows:Cd: Leaves > Stems > Seeds. As: Stems >Leaves>Seeds. In a decreasing order of translocation from the roots to the aerial parts of *H. annuus* .

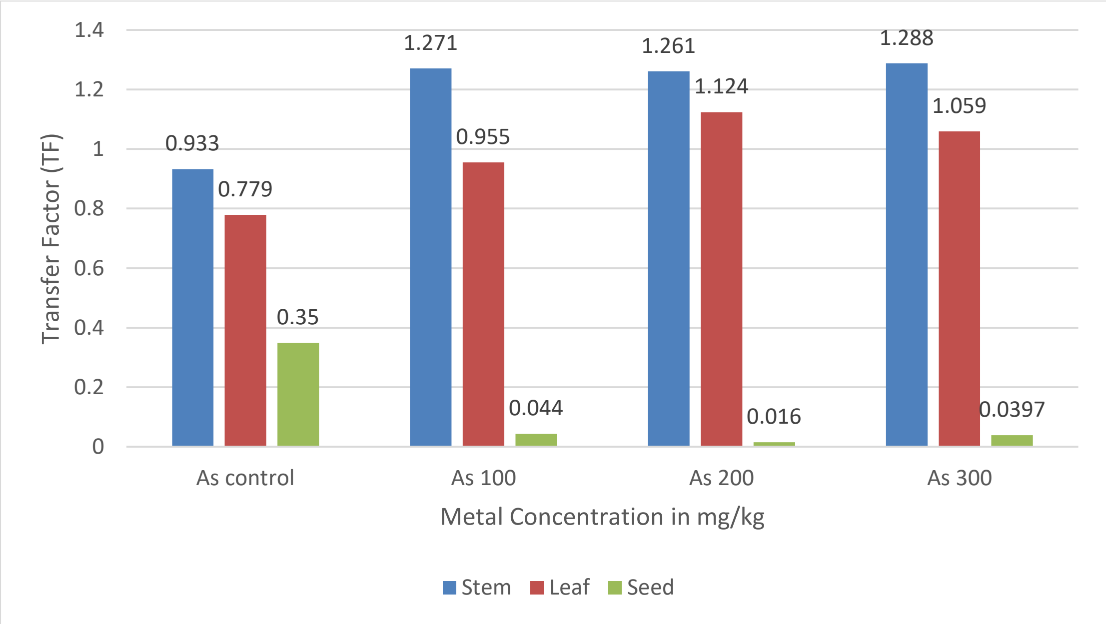

Figure 1.3: Transfer Factor of As in Plant Part of *H. annuus*

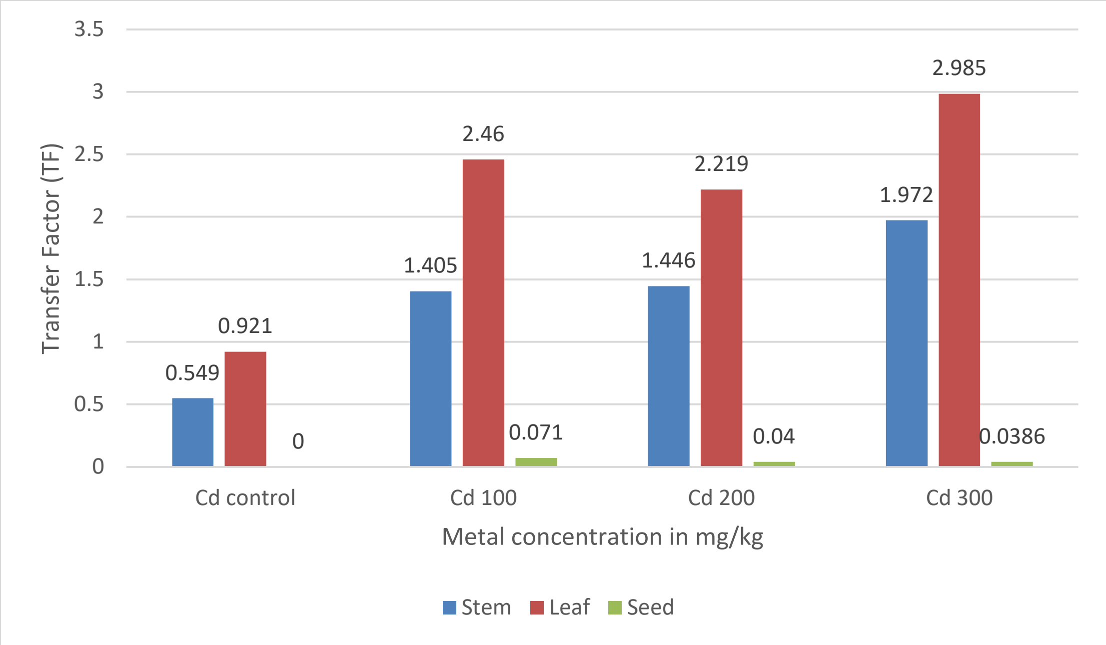

Figure 1.4: Transfer Factor of cadmium in plant parts of *Helianthus annuus*

Discussion

Assesment of the Physico-Chemical quality and levels of heavy metals content in the Soil.

Soil-property, speciation of heavy metal, bioavialabilty and the specie of the plant concerned or the selection of plant specie for phytoextraction of heavy metals are key factors associated with phytoremediation of contaminated soil environment.Hence, a better understanding of interactions among the four key factors in the rhizosphere namely; soils, metals, microbes and plant roots are of absolute necessity,thereare this study had examined the physicochemical characteristics of the experimental soils, the obtained result of the Physio-chemical characteristic of the experimental soil are presented in table 1.1. The percentage sand, silt and clay are 72.78, 10.11 and 1.85 (%) respectively indicated the textural class to be loamy soil due to its richness in organic matter. The P^H- (5.183) indicated an acidic reaction of the soil, which is a measure of the activity of H^+in the soil solution having ranged strongly between (P^H 5.5 – 45) to extreme acidity (P^H< 4.5). bioavailability and mobility of heavy metal contents of the soil was known to increase by the lowering soil P^H of (5.183), since metal salts are soluble in acidic media rather than in basic media. The soil total carbon and nitrogen of 2.039 and 3.3167 respectively was indicative of mineralization rather than microbial immobilization which therefore means microbial activity in soil and release the nitrogen and phosphorus available for plant up take. Preliminary analysis of the soil samples and control-soil- were detected with no organic chlorination, pesticides and fungicides. Other parameters such as base saturation CEC, EC and EA were in the limit value of 6.8cmol/kg, 2.03 dsm and 1.48 Cmol/kg for sensitive soil. The result also presented values for heavy metals As and Cd concentration detected in the soil samples before planting of the experimental plants as follows: As: 0.9593mg/kg ± 0.0164; Cd: 5.1410mg/kg ± 0.02436. this is attributed to the fact that soil are not totally free of heavy metals but may be mechanically bound in its lattice orunavailable form for plant uptake.

Concentration levels of heavy metals in parts of *H.annuus*

Sunflower (*H. annuus*) leaf – Pb content was higher than leaf – Cd content by mean value of 106.3433 .As trail behind with lowest concentration was recorded in control seed (0.000 ± 0.00000) in the following order: Cd>As. In a decreasing order of concentration as per the plant parts as follows: Leaf>Stem>Root>Seed at a decreasing sequence of concentration levels as follows: 300mg/kg> 200mg/kg> 100mg/kg > 0mg/kg.

Evaluation of phytoremediation parameters (Bioconcentration factor (BCF) and Transfer Factor (TF) in the plant under investigation

Bioconcentration factor

Data revealed, the BCF of different parts of *H. annuus* with its corresponding levels of metal contamination to be highest in leaves with BCF of 0.3629 grown on As contaminated soil while Cd accumulated more in the stem, the distribution of the heavy metals in organs of *H. annuus* in decreasing order as follows:

As:	Leaves>	Stem	>	Root	>	Seed
Cd:	Stem >	Leaves	>	Root	>	Seed

Transfer Factor

Yadav et al, 2009 observed that plant with TF values>1 are classified as high efficiency plant for metal translocation from roots to shoots and as such could be considered as good phytoremediators. Result shows the plant ability to translocate heavy metal contents from the roots to the shoots systems, can be classified as high-efficiency plants for metal translocation from roots to shoots as translocation factors of the examined metals for the tested plant are above TF values > 1. The author observed that the levels of metals in roots and stems after 20 weeks of plants growth had more bio availability pool of test metals translocated from roots to seed< leaves < stem in increasing order. While roots to seed transfer was in negligible amount and was below the WHO/FAO limit for edible plant parts.

Conclusion

The general trend of heavy metal accumulation in plants parts of *H. annus* in terms of Bio concentration factors indicated that plant is an hyper-accumulator of heavy metals having exceeded BCF bench mark of 1 > 10.Figure 1.1 -1.4 shows the plants ability to translocate heavy metal content from the root to shoot systems clearlyand therefore classified as high efficiency plants for metal translocation from root to shoot as the transfactors are above TF value > 1. Therefore phytomediation is a good technology for removal of heavy metals and metalloids in polluted soil environment.

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