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HEAVY METAL ACCUMULATION POTENTIAL OF BARLEY (*HORDEUM VULGARE*)

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ABSTRACT

This research was aimed at assessing the metal accumulation potential of the grass, *Hordeum vulgare* for effective phytoremediation. Laboratory pot experiment was conducted. Seed of the grass were seeded into 2.0 kg soil spiked with different concentrations of, 150, 500 and 1000ppm for Pb and Ni as $Pb(NO_3)_2$ and $Ni(NO_3)_2.6H_2O$; 150, 250, and 400ppm for Cd as $Cd(NO_3)_2$; 150, 500 and 1000ppm for Se, as SeO_2. Experiment was monitored with adequate irrigation for a period of eight weeks with the control. At the end, harvested plants were separated into roots and shoots, treated and analyzed along with the soil using atomic absorption spectrophotometer. The enrichment (EF) and translocation factors (TF) of the metals were calculated. The results showed that, lead (Pb) has the TF values of 0.35, 0.85, 0.57 at the different concentrations respectively and 0.81 for the control. Its EF values are; 1.23, 1.02, 4.93 and 2.62 for the control respectively. Cadmium has the TF values of 0.82, 0.71, 1.11 respectively and 1.01 for the control. Its EF values are 0.88, 0.75, 0.97 respectively and 1.02 for the control. Nickel has 0.32, 0.42, 0.40 respectively and 0.86 for the control. Its EF values are; 0.59, 1.33, 1.17 respectively and 1.19 for the control. Selenium on the hand has the TF values of 1.45, 0.63, 0.58 and 1.01 for the control. These TF and EF values suggest that, *Hordeum vulgare*, may serve as a soil stabilizer for Pb, Ni and Se; one of the techniques of phytoremediation known as phytostabilization for having TF values less than one (1), and EF values greater than one (1) at most of the different

concentrations. For cadmium, the control and at 400ppm Cd in the pot, has the TF value greater than one (1) and the EF values were all less than one which shows that Cd is stored at high level in the harvestable parts of the plant, the shoot. Thus can be harvested and disposed. The plant may therefore be defined as Cd phytoextractor. Phytoextraction, is also a technique of phytoremediation which involves the uptake and accumulation of contaminants in the shoot of plant species at higher level than the root without sign of toxicity.

KEYWORDS: Environment, heavy metal, soil, contamination, phytoremediation.

INTRODUCTION

The quality of life on Earth is linked extensively to the overall quality of the environment. The major functions of a soil are generally recognized to include the ability to protect water and air quality, the ability to sustain plant and animal productivity, and the ability to promote human health (Garba et al., 2017a). Contaminated soils and residues therefore, can be remediated by various methods, such as: removal, isolation, incineration, solidification/stabilization, vitrification, thermal treatment, solvent extraction, chemical oxidation etc., to improve its quality. These methods have the disadvantage of being very expensive and in some cases, they involve the movement of contaminated materials to treatment sites thus, adding risks of secondary contamination (Evanko and Dzombak, 1997; Prasad, 2004). Currently emphasis is being given to in situ methods that are less environmentally disruptive and more economical. The knowledge of the mechanism of uptake, transport, tolerance and exclusion of heavy metals and other potentially hazardous contaminants by microorganisms and plants have recently promoted the development of a new technology, referred to as, bioremediation (Garba et al., 2017a). It is based on the potentials of living organisms, mainly microorganisms and plants, to detoxify the environment. Plant based bioremediation technologies have been collectively termed as phytoremediation, a suitable alternative to the conventional methods.

Phytoremediation harnesses natural processes to assist in the clean-up of pollutants in the environment. It takes the advantage of the unique and selective uptake mechanisms by plants (trees, shrubs, grasses and aquatic plants) and their associated microorganisms in order to remove, degrade or isolate toxic substances like heavy metals, trace elements, organic compounds and radioactive compounds from the environment (Prasad and Freitas, 2003; Dickinson et al., 2009). The word “phytoremediation” derives from the Greek «phyton», meaning “plant”, and Latin «remedium», which means “to remedy” or “to correct”.

Phytoremediation techniques include different modalities, depending on the chemical nature and properties of the contaminant (if it is inert, volatile or subject to degradation in the plant or in the soil) and the plant characteristics. Certain plants have been identified not only to accumulate metals in the plant roots, but also to translocate the accumulated metals from the root to the leaf and to the shoot. While many plants performed this function, some plants, known as “hyperaccumulators”, can accumulate extremely high concentrations of metals in their shoots (0.1% to 3% of their dry weight) (Huang and Cunningham, 1996). The metal-rich plant material can then be harvested and removed from the site without extensive excavation, disposal costs, and loss of topsoil that is associated with traditional remediation practices. Thus, phytoremediation essentially comprises six different strategies, though more than one may be used by the plant simultaneously (Figure 1).

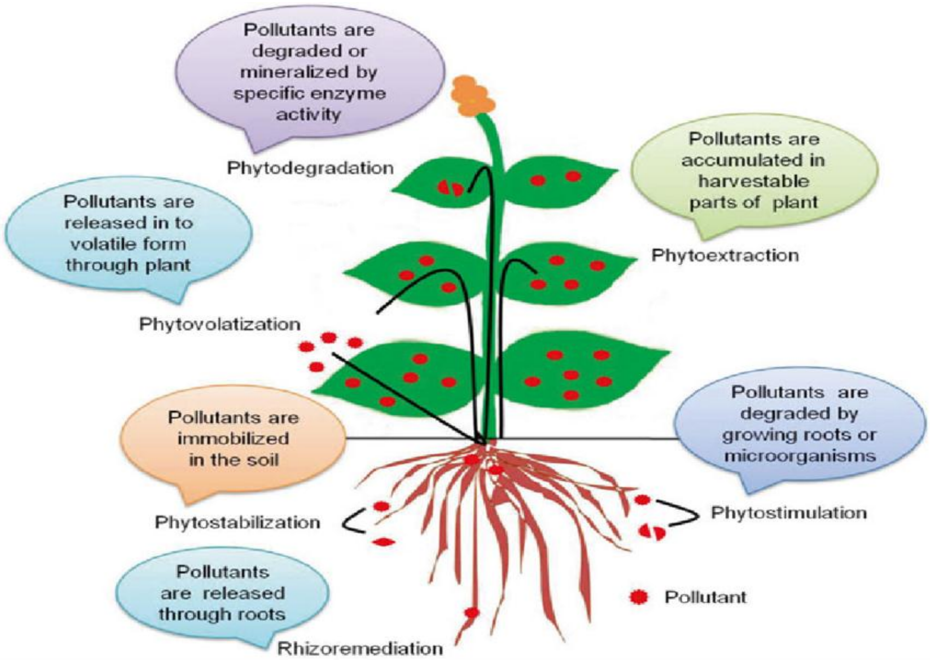

Figure 1: Schematic diagram of different approaches of phytoremediation.

The success of phytoremediation however, is dependent upon several factors. No plant has been discovered yet capable of meeting all the ideal criteria of an effective phytoremediator. These criteria are, plants must produce sufficient biomass while accumulating high concentrations of contaminants (especially heavy metals). In some cases, an increased biomass will lower the total concentration of the metal in the plant tissue, but allows for a larger amount of metal to be accumulated overall. The metal-accumulating plants need to be responsive to agricultural practices that allow repeated planting and harvesting of the metal-rich tissues. Thus, it is preferable to have the metal accumulated in the shoots as opposed to the roots, for metal in the shoot can be cut from the plant and removed. This is manageable on a small scale, but impractical on a large scale. If the metals are concentrated in the roots, the entire plant needs to be removed. Yet, the necessity of full plant removal not only increases the costs of phytoremediation, due to the need for additional labor and plantings, but also increases the time it takes for the new plants to establish themselves in the environment and begin accumulation of metals. The availability of metals in the soil for plant uptake is another limitation for successful phytoremediation (Paz-Alberto and Sigua, 2013). The selection of promising plants is an important approach to successful phytoremediation.

The plants used for phytoremediation procedures can range from those with natural ability, moderate accumulator to hyper-accumulator or those that degrade or render harmless contaminant in soils, water and air. It is a highly technical strategy, that requires expert project designers with field experience that choose the proper species and cultivars for particular metals and regions (Alkorta et al., 2004). One or a combination of these plants is selected and planted at a site based on the type of metals present and other site conditions (Rajkumar et al., 2012). After the plants have been allowed to grow for several weeks or months, they are harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits. Some studies have identified grasses as potential phytoremediators (Pichtel and Liskanen, 2001; Siddiqui and Adams, 2002; Kim et al., 2006). Thus, the assessment of native site-specific grasses is recommended for a better understanding of the phytoremediation potential for each particular, site-specific situation. The objectives of this study are; 1) to determine the survival rate and vegetative characteristics of the grass specie Barley (*Hordeum vulgare*), grown in soils amended with different levels of; Cd, Ni, Se and Pb; 2) to determine the accumulation ability of the grass specie for effective phytoremediation of the selected metals.

MATERIALS AND METHODS

Sample Collection

Viable seeds of the grass, *Hordeum vulgare* were collected from the plants dried husks. The soil that supported the growth of the grass was equally collected from the surface to subsurface portions, just beneath the roots of the grass. Samples were collected from Lake Chad Research Institute situated at Km 5 Gamboru Ngala Road Maiduguri, Nigeria.

Laboratory pots experiment

Pot culture experiment was conducted using 2.0 kg soil spiked with the soluble salt of the metals Cd, Ni, Se and Pb. Experimental soil was spiked with the salt of Ni as $Ni(NO_3)_2.6H_2O$, Se as SeO_2, Pb as $Pb(NO_3)_2$ and Cd as $Cd(NO_3)$ at a concentration of 150ppm, 250pmm, 400ppm for Cd and Se; 150, 500, and 1000ppm was for Pb and Ni respectively. Viable seeds of the grass, were sawn into the pots. Separate pots containing the same amount of untreated soil (2 kg) was equally seeded to serve as a control (Garba et al., 2011). Experiments were exposed to natural day light and night temperatures, and since humidity is one of the factors ensuring the growth of plants and the necessary physiological processes, irrigation of the pots was done with 500 ml of water after every five days in the evening hours. Plastics trays were place under each pot and the leached was collected and put back in their respective pots in other to prevent loss of nutrients and trace element from the samples (Garba et al., 2011). The grasses were allowed to grow for a period of eight weeks and harvested to avoid loss of accumulated metals through the shedding of vegetative parts or poor uptake due to age. Four replicates of experimental pots for each element was seeded for statistical handlings.

Statistical Data Handling

All statistical data handling was performed using SPSS 12 package. The difference in mean of heavy metal concentration among the different samples was detected using one-way ANOVA, followed by multiple comparisons using Tukey test. A significant level of ($P = 0.05$) was considered throughout the analysis.

RESULTS AND DISCUSSION

The physicochemical properties of the experimental soil are as shown in table 1 below. The taxonomy classification of the soil was found to be sandy loam with pH of (6.27). The less acidic nature of the soil is generally within the range for soil in the region; soil pH plays an important role in the sorption of heavy metals, it controls the solubility and hydrolysis of metal hydroxide, carbonate and phosphates (Garba et al., 2018). A very low organic carbon was observed in the soil sample (0.53) which led to the low organic matter content observed (0.90) as well as low cation exchange capacity (CEC) (4.09 mol/100kg soil). CEC measure the ability of soil to allow for easy exchange of cations between it surface and soil. The low level of clay and CEC indicate the permeability and leachability of metals in the soil.

Appreciable amount of silt was observed in the soil sample (20.70), silt improves the soil, resulting in better plant growth.

Table 1: The physicochemical properties of the experimental soil.

Parameters	Soil
pH	6.27 ±0.004
EC (dsm^{-1})	0.38 ±0.006
CEC (mol/100kg soil)	4.09 ±0.007
Organic Carbon (%)	0.53 ±0.005
Organic Matter Content (%)	0.91 ±0.005
Silt (%)	20.70 ±0.006
Sand (%)	14.70 ±0.005
Textural Class	Sandy loam

Data are presented in mean ± standard deviation (SD) with n = 3

Lead (Pb)

Lead is a major pollutant in both terrestrial and aquatic eco-system. Besides natural weathering processes the main sources of Pb pollution are exhaust fumes of automobiles, chimneys of factories using Pb, effluents from the storage battery, industry, mining and smelting of Pb ores, metal plating and finishing operations, fertilizers, pesticide and additives in pigments and gasoline (Eick et al., 1999). The main pathway by which plants accumulate metals is through root uptake from soils (Sharma and Dubey 2005; Uzu et al. 2009). Although, lead uptake is a non-selective phenomenon, it nonetheless depends on the functioning of an H^+/ATPase pump to maintain a strong negative membrane potential in rhizoderm cells (Hirsch et al. 1998; Wang et al. 2007). Once lead has penetrated into the root system, it may accumulate there or may be translocated to aerial plant parts. For most plant species, the majority of absorbed lead (approximately 95% or more) is accumulated in the roots, and only a small fraction is translocated to aerial plant parts, as has been reported in *Vicia faba*, *Pisum sativum*, and *Phaseolus vulgaris* (Piechalak et al. 2002; Małecka et al. 2008; Shahid et al. 2011; Garba et al., 2017a).

These observations agree with the findings of this study, high level of Pb was absorbed and accumulated in the root. The level of uptake and accumulation increases as the concentration of the metal in the soil increases (Table 2). There are several reasons why the transport of lead from roots to aerial plant parts is limited. These reasons include immobilization by negatively charged pectins within the cell wall (Islam et al. 2007; Arias et al. 2010), precipitation of insoluble lead salts in intercellular spaces (Kopittke et al. 2007; Małecka et al. 2008), accumulation in plasma membranes (Seregin et al. 2004; Jiang and Liu 2010), or sequestration in the vacuoles of rhizodermal and cortical cells (Seregin et al. 2004; Kopittke et al. 2007). However, these reasons are not sufficient to explain the low rate of lead translocation from root to shoot. Several hyperaccumulator plant species, such as Brassica pekinensis and Pelargonium, are capable of translocating higher concentrations of lead to aerial plant parts, without incurring damage to their basic metabolic functions (Xiong et al. 2006; Arshad et al. 2008).

Toxic Effect of Pb on the Growth of the Plant

Several toxic effect on the germination and growth of plants has been attributed to Pb. Report has it that, germination is strongly inhibited by even very low concentrations of Pb^{2+} (Tomulescu et al. 2004). Lead exposure in plants also strongly limits the development and sprouting of seedlings (Dey et al. 2007; Gopal and Rizvi, 2008). At low concentrations, lead inhibits the growth of roots and aerial plant parts (Islam et al. 2007; Kopittke et al. 2007). Under severe lead toxicity stress, plants displayed obvious symptoms of growth inhibition, with fewer, smaller, and more brittle leaves having dark purplish abaxial surfaces (Islam et al. 2007; Gupta et al. 2009). Lead-induced inhibition of seed germination has been reported in *Hordeum vulgare, Elsholtzia argyi, Spartina alterniflora, Pinus halepensis, Oryza sativa*, and *Z. mays* (Tomulescu et al. 2004; Sengar et al. 2009). In this study however, smooth germination was observed. No noticeable symptoms were observed on germination and growth of the experimental plants (Figure 1) compare to the control (Figure 7). Report has it that, at higher concentrations, lead may speed up germination (Islam et al. 2007).

Table 2: Levels (ppm) of Pb in Soil, Shoot, Root of *Hordeum vulgare*.

Amount Spiked	Soil	Root	Shoot
150	156 ±0.004	546 ± 1.000	192 ±0.004
500	647 ±0.004	779 ± 0.005	659 ±0.003
1000	102 ±0.006	891 ±0.003	503 ±0.005
Control	137 ± 0.011	446 ± 0.003	359 ± 0.007

Data are presented in mean and ± Standard Deviation (SD), means were found not significant at P = .05 using one-way ANOVA and multiple comparison (Post-Hoc) according to Tukey test.

Figure 1: The Growth of *H. vulgare* in the Experimental Pots Spiked with Different levels of Pb.

Cadmium (Cd)

Cadmium is a trace element with unknown essential functions for plants. It is, however, readily absorbed by plant roots and translocated to above-ground parts. Cadmium concentrations (dry weight-based) are typically higher in the plant leaves than in fruits or storage organs. The uptake of Cd by plant increases proportionally to increasing soil Cd when soil contains substantial concentration of Cd^{2+} salts (Smolders, 2001). In this study, the uptake and accumulation of Cd by the plant, *Hordeum vulgare,* is as shown in table 3. The result showed that, slightly high level of the metal is retained in the root compare to the shoot. This trend was maintained (high level of the metal retained in the root) as the concentration of Cd in the soil increases although with slight decrease in the level accumulated compare to the control (Table 3). Lozano-Rodrfguez et al. (1997) reported that, total Cd concentration of shoot and root in maize and pea plants increased concurrently with the treatments applied and its accumulation being approximately 10 times higher in root than in shoot. However, in this study, as the concentration of Cd in soil medium increases to 400 ppm, the pattern of accumulation changes. The shoot was found to accumulate high level of the metal than the root (Table 3). Higher shoot Cd accumulation in bread wheat cultivar has been reported, this, reflects its differential distribution between roots and shoots, and not as a result of slightly greater uptake by bread wheat roots (Hart el et al., 1998).

Translocation of Cd from root to shoot has been studied in several species, including ryegrass *Secale cereal*, (Jarvis *et al*., 1976), tomato (*Lycopersicon esculentum*; Petit and vande Geijn, 1978), bean (*Phaseolus vulgaris*; Hardiman and Jacoby, 1984), maize (Yang et al., 1995), and durum wheat (Jalil et al., 1994). Movement of Cd from roots to shoots is likely to occur via the xylem and to be driven by transpiration from the leaves. However, the accumulation of Cd in the shoots of plants is generally dependent on the roots as its primary source (John et al., 2008). Despite the difference in mobility of the metal ions in the plants the metal content is generally greater in the root than in the above-ground tissues (Ramas et al., 2002). Most Cd ions are retained in the roots and only small amounts are transported to shoots (Cataldo et al., 1983) as is the case in this study (Table 3). It has been reported that, the concentration of Cd in plants decreases in the order: root > leaves > fruits > seeds (Blum, 1997; Sharma et al., 2006).

Toxic Effect of Cadmium on the Plant Growth

Cadmium is not an essential nutrient and at high concentration inhibits plant growth (Anita et al., 1990; Aery and Rana, 2003). No sign of toxicity of Cd was observed on the experimental plants (Figure 2) when compared with the control (Figure 7) of this study, reduction in growth has been associated with cadmium treatment which was reported to caused inhibition of protein synthesis (Foy et al., 1978). It has also been reported that even at relatively low concentrations it alters plant metabolism (Van Assche and Clijsters, 1990). The presence of cadmium in the soil has been observed to decrease the growth of soybean (Dewdy and Ham, 1997, Cataldo et al., 1983) and chickpea plants (Hasan et al., 2007). High concentrations of Cd decreased cell growth as well as whole plant growth (Prasad, 1995).

Table 3: Levels (ppm) of Cd in Soil, Shoot, Root of *Hordeum vulgare*.

Amount spiked	Soil	Root	Shoot
150	369.00 ± 0.0001	393.00 ±0.001	324.00 ±0.005
250	371.00 ± 0.0008	386.00 ±0.0007	277.00 ±0.0008
400	376.00 ±0.007	328.00 ±0.002	365.00 ±0.0012
Control	390.00 ±0.0018	398.00 ±0.0013	396.00 ±0.0011

Data are presented in mean and ± Standard Deviation (SD), means were found not significant at P = .05 using one-way ANOVA and multiple comparison (Post-Hoc) according to Tukey test.

Figure 2: The Growth of *H*. v*ulgare* in the Experimental Pots Spiked with Different levels of Cd.

Nickel (Ni)

In nature, Ni is mostly present in the form of nickelous ion, Ni^{2+}. The hydrated form as Ni $(H_2O)_6^{2+}$, is the most common form of Ni found in the soil solution. It also occurs in water bodies and in other atmospheres, usually in trace amounts. The release of municipal and industrial effluents significantly contributes Ni content to the soil and water (Yusuf et al., 2011). The uptake of Ni in plants is mainly carried out through the root system via passive diffusion and active transport (Seregin and Kozhevnikova 2006). The ratio of uptake between active and passive transport varies with the species, form of Ni and concentration in the soil or nutrient solution (Dan et al. 2002; Vogel-Mikus et al. 2005). The overall uptake of Ni by plants depends on the concentration of Ni^{2+}, plant metabolism, the acidity of soil or solution, the presence of other metals and organic matter composition (Chen et al. 2009). However, uptake of Ni usually declines at higher pH values of the soil solution due to the formation of less soluble complexes (Temp 1991). Besides being absorbed by roots, Ni can also enter into the plants via leaves (Sajwan et al. 1996; Hirai et al., 1993). The path of Ni transport in plants is from root to shoot (Peralta-Videaa et al. 2002) and makes an exit through transpiration stream (Neumann and Chamel 1986) via xylem.

Survey of literature reveals that distribution of Ni in plant tissues mainly deals with its localization in the shoots of hyperaccumulator plant species (Heath et al. 1997; Bhatia et al. 2004). In this study, much of the metal (Ni) absorbed were observed to accumulate in the root (Table 4). Marques et al. (2009) reported that in *Rubus ulmifolius,* Ni was only distributed in the root. Cataldo et al. (1978) reported that over 50% of Ni absorbed by plants is retained in the roots. This may be due to the sequestration in the cation exchange sites of the walls of xylem parenchyma cells and immobilization in the vacuoles of the roots (Seregin and Kozhevnikova 2006). The observation made in this study agree with the report that, Ni accumulation was more pronounced in roots rather than the shoot in barley (Brune and Deitz 1995) and maize (Baccouch et al. 2001). As the uptake of Ni predominates via roots, it is of primary importance to unravel the pattern of Ni distribution in the underground organs.

Toxic Effect of Ni on the Plant Growth

Nickel showed no visible phenotypical changes at different spiked concentration of, 150ppm, 500ppm and 1000ppm Ni on the experimental plants. Rather uniform growth rate was observed (Figure 3) when compared with the control (Figure 7). Nickel has been classified as one among the essential micro nutrients and remains associated with some metallo-enzymes. Browen et al. (1987) have demonstrated that Ni is an essential micronutrient for *H.* v*ulgare* which was observed not to complete its life cycle in the absence of Ni and addition of Ni to the growth medium completely alleviated its deficiency symptoms. Although Rahman et al. (2005), reported foliar chlorosis and necrosis in barley grown in 0.1mM Ni for 14 days. However, presence of excess Ni in the external environment has been reported to cause some changes in the growth pattern and development of some plants. These effects are summarized in table 5 (Yusuf et al., 2011). The impact of Ni toxicity on the physiology of plants has been envisaged to depend on the type of plant species, growth stage, cultivation conditions, Ni concentration and exposure time (Marschner 1995; Kabata-Pendias and Pendias 2001; Assuncao et al. 2003) in the soil.

Table 4: Levels (ppm) of Ni in Soil, Shoot, Root of *Hordeum vulgare.*

Amount spiked	Soil	Root	Shoot
150	37.00 ±1.000	68.50 ±1.000	22.00 ±0.002
500	39.00 ±0.005	123.00 ±0.003	52.00 ±1.000
1000	114.50 ±0.002	338.00 ±0.006	134.00 ±0.002
Control	8.00 ±1.000	11.00 ±0.001	9.00 ±1.000

Data are presented in mean and ± Standard Deviation (SD), means were found not significant at P = .05 using one-way ANOVA and multiple comparison (Post-Hoc) according to Tukey test for.

Figure 3: The Growth of *H. vulgare* in the Experimental Pots Spiked with Different levels of Ni.

Table 5: Effect of Nickel on Stem Growth in Different Plants.

Nickel concentration	**Crop/plant**	**Effect**	**Reference**
100 µM $NiSO_4$	*Triticum aestivum*	Decrease mesophyll thickness, size of vascular bundle, and width of epidermal ells	Kovaccevic et al. 1999
200 µM Ni	Wheat seedlings	Lowered shoot length by 44%	Gajewska et al. 2006
> 50 mM Ni	Soybean seedlings	Decrease the fresh and dry mass of the plant	El-Shintinawy and El-Ansary 2000
100 µM Ni	*Triticum aestivum*	Reduced shoot growth appearance of chlorosis and necrosis	Gajewska and Sklodowska 2007

Selenium (Se)

In a global context, selenium (Se) is a complex but interesting element. The boundaries between animal toxicity and deficiency of Se are relatively narrow, and both phenomena are common around the globe (Haygarth, 1994). The uptake, translocation and distribution of Se depends upon plant species, phases of development, form and concentration of Se, physiological conditions (salinity and soil pH) and presence of other substances, activity of membrane transporters, translocation mechanisms of plant (Zhao et al., 2005; Li et al., 2008; Renkema et al., 2012). Selenate (SeO_{2-4}) is the most prevalent form of bioavailable Se in agricultural soils, and more water soluble than selenite (Sors et al., 2005; Missana et al., 2009). In alkaline soils, Se mostly exists as selenate whereas, in acidic soils it exists as selenite. Both forms of Se differ in terms of their mobility and absorption within the plant and are metabolized to form selenocompounds (Li et al., 2008). Most plants accumulate more Se in shoot than in the root tissues, but there are exceptions (Zayed et al., 1998). For instance, Se contents of five samples of Verbascum Thapsus (VR) were found to be 0.74, and 0.50mgkg^{-1}, for root and shoot respectively. Similarly, the Se content of roots, and shoots of Isatis (IS), were found to be 0.50, and 0.48 mgkg^{-1} Se, respectively (Sasmaz et al., 2015).

In this study, high level of the metal, Se, was observed in the shoot in the control and when the level of the element in the experimental pot was 150ppm (Table 6). The pattern of accumulation however, changes when the concentration in the soil was increased to 250 and 400ppm respectively. High concentration of the element was observed in the root compare to the shoot (Table 6). Report has it that, majority of plants accumulate more Se in shoot and leaf than in root tissues, but there are exceptions (Zayed et al., 1998). The transport of Se from roots to shoots is considered to occur via the xylem. Plants transport selenate to leaves where they accumulate substantial amounts, but much less selenite or selenomethionine is stored. Selenite is rapidly reduced to organic forms of Se (selenomethionine) in plants which is retained in the roots (Terry et al. 2000; Sors et al. 2005). According to Terry and Zayed (1994), the absorption and translocation of selenate in plants is believed to resemble closely the uptake and movement of sulfate. A five-fold increase in sulfate (from 33mgSL-' with selenate at 0.1, 0.5, or 1.0 mgSeL^{-1}) produced a six-fold decrease in the concentration of Se in shoots of barley (cv. Briggs) after 28 days in solution culture; similarly, a two-fold increase in sulfate (from 16 mg S L^{-I} with selenate at 0.5 mg Se L^{-I}) produced a two-fold decrease in the concentration of Se in shoots of rice (cv. M101) after 60 days (Mikkelsen et al., 1990). The increase in the level of Se in experimental pot might triggered the absorption of which subsequently reduces the translocation of Se to the shoots.

Toxic Effect of Se on the Plant Growth

Selenium is not considered to be an essential element for flowering plants (angiosperms), although it is considered to be a beneficial element since it can stimulate growth, confer tolerance to environmental factors inducing oxidative stress, and provide resistance to pathogens and herbivory (Quinn *et al*., 2007; White and Brown, 2010; Feng et al., 2013). Similarly, no sign of toxicity was observed on the plant in course of germination as well as growth (Figure 6) compare to the control experiment (Figure 7). Selenium toxicity occurs in plants when optimum concentration of Se exceeds. Selenium causes toxicity by two mechanisms, one of which is malformed selenoproteins (Pilon-Smits et al., 2002; Hondal et al., 2012) and another by inducing oxidative stress (Hugouvieux et al., 2009; Lehotai et al., 2012). Both the mechanisms are known to be harmful for plants in one or other way. Report has it that, non-accumulators are sensitive to high Se concentration, they can tolerate as well as accumulate even high concentrations of Se without growth reduction when grown in Se-enriched soils (Rani et al., 2005).

Table 6: Levels (ppm) of Se in Soil, Shoot, Root of *Hordeum vulgare.*

Amount spiked	Soil	Root	Shoot
150	224.00 ± 0.058	249.00 ±0.052	360.00 ±0.117
250	175.00 ± 0.123	471.00 ±0.070	298.00 ±0.142
400	332.00 ±0.050	455.00 ±0.071	262.00 ±0.020
Control	33.00 ±0.086	94.00 ±0.134	95.00 ±0.035

Data are presented in mean and ± Standard Deviation (SD), means were found not significant at P = .05 using one-way ANOVA and multiple comparison (Post-Hoc) according to Tukey test.

Figure 6: The Growth of *H. vulgare* in the experimental pots spiked with different levels of Se.

Figure 7: *H. vulgare* in the control experimental pot.

Phytoremediation Potential of the Plants, *Hordeum. vulgare*

The levels of metals in different parts of plants especially the root, stem and the leaves does not simply predict the phytoremediation potentials of such plants. The values of translocation (TF) and enrichment (EF) factors calculated from the concentrations of the elements from ratio of the root or shoot and the soil determine the phytoremediation ability of plants (Garba et al., 2017b). Bioaccumulation factor also called bioconcentration factor (BCF) is used in the determination of the degree of intake and component storage of toxic compounds in plants and animals (Connell, 1997). It refers to the ratio of plant metal concentration in roots tissues to the soil or polluted environment [(Metal) root/ (Metal) polluted environment or substrate].

Enrichment Factor (EF) and Translocation Factor (TF)

Several studies (Baker, 1981; Yoon et al., 2006; Srivastava et al., 2006) have envisaged that, the ability of phytoremediation has commonly been characterized by a translocation factors (TF). According to MacFarlane et al. (2007), translocation factor (TF) is defined as the ratio of concentration of metals in the shoot or above ground parts of plants to the metal concentration in the roots.

$$\text{TF} = \frac{Concentration\ of\ metal\ in\ the\ shoot}{Concentration\ of\ metal\ in\ the\ root}$$

In this study, the TF values for the elements; Cd, Se, Pb and Ni presented in the figure eight (8), indicating the uptake and accumulating ability of the plant for phytoremediation. Plants with TF values of one (1) and above are classified as high-efficiency plants for metal translocation from the roots to shoots (Ma et al., 2001). The identification of metal hyperaccumulators, plants capable of accumulating extra ordinary high metal levels in the above ground tissues, demonstrates that plants have the genetic potential to clean up contaminated soil. Hyperaccumulators are characterized by the translocation factor of one (1) and above.

Enrichment Factor (EF)

Enrichment factor (EF) is been calculated to derive the degree of soil contamination and heavy metal accumulation in soil and in plants growing on contaminated site with respect to soil and plants growing on uncontaminated soil (Kisku et al., 2000). It is at considered as an indicator used to assess the presence and intensity of anthropogenic contaminant deposition on surface soil (Balls et al., 1997). Enrichment factor is calculated as the ratio plant shoot metal concentration to contaminated environmental medium (e.g. soil and wastewater) concentration [(Metal) shoot/ (Metal) polluted substrate (Branquinho et al., 2007).

$$EF = \frac{Concentration\ of\ metal\ in\ the\ shoot}{Concentration\ of\ metal\ in\ polluted\ environment\ (soil\ or\ water)}$$

In this study, the EF values for the elements; Cd, Se, Pb and Ni presented in the figure eight (8), indicating the level of contamination of the soil and accumulating ability of the plant mostly in the root zone.

The result indicated that, the TF Value for Pb at 150ppm is 0.35; at 500ppm is 0.85; at 1000ppm is 0.57 whereas the control has a TF Value of 0.805. The EF values are, 1.23, 1.02, 4.93 and 2.62 at 150ppm, 500, 1000ppm and the control respectively. All the BCF values just like the EF values are greater than one (1) (Figure 8). For having TF values less than one (1), and EF values greater than one (1), the plant *H. vulgare* has the ability to absorb and retain or accumulate the metal Pb in the root zone. A process known as phytostabilization. Heavy metal-tolerant species with high EF (greater than one) and low TF values (less than one) can be used for phytostabilization of the metals within the root zone in the soil (Garba et al., 2017b). It is one of the techniques of phytoremediation. The BCF values greater than one (1) indicated high degree of absorption by the plant.

For cadmium, the TF values at 150ppm is 0.82; at 250ppm is 0.71; at 400ppm is 1.11 whereas the control has TF value of 1.01 (Figure 8). It has the EF value of 0.88, 0.75, 0.97 and the control 1.02. This shows that, *H. vulgare* can absorb and translocation the metal Cd to the above ground tissues (shoot). It also indicated that, translocation to shoot, of Cd, is greater when the level of the metal in the soil is high. The TF value is greater than one for the control as well as at 400ppm which shows that the metal Cd is stored in the shoot gradually. Phytoextraction one of the process of phytoremediation, usually involves the uptake of toxic heavy metals from contaminated soils and their accumulation in harvestable parts of plant species. Plants being considered as hyperaccumulators must have the potential to tolerate the metals and transfer them from roots to above-ground parts of the plant species (Blaylock and Huang, 2005). One of the important factors affecting the success of phytoremediation of Cd-polluted soils is the availability of high biomass plants with the ability to concentrate Cd to high levels within their shoots.

The TF Value for Ni at150ppm is 0.32; at 500ppm is 0.42; and at 1000ppm is 0.40 whereas the control has TF value of 0.86 as shown in figure 8. The EF are 0.59, 1.33, 1.17 at 150ppm, 500 and 1000ppm whereas the control has 1.19. For Ni, it is the EF values that are greater than one (1), the TF values are all less than one (1), these indicate that the plant *H. vulgare* has the ability to absorb and accumulate the metal Ni in the root zone. Enrichment factors greater than one suggest that, the plant can stabilize or accumulate the metal in the root zone rather than to mob the soil of the metal. A process known as phytostabilization. It is mostly used for the remediation of soil, sediment and sludges (Mueller et al., 1999) and depends on roots ability to limit contaminant mobility and bioavailability in the soil. Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction. Base on the EF values for Ni, *H. vulgare* may be use to stabilize the soil.

For selenium, the TF Values at 150ppm is 1.45; at 250ppm is 0.63; at 400ppm is 0.58 whereas the control has the TF value of 1.01 (Figure 8), and the EF values are 1.61, 1.71, 0.79, and 0.28 at the three different spiked level of the metal in the soil (150, 250, 400ppm) and the control. The plant, *H. vulgare* can therefore best be defined as a stabilizer for Se in the soil (Figure 8). This is attributed to greater value of the EF than the TF.

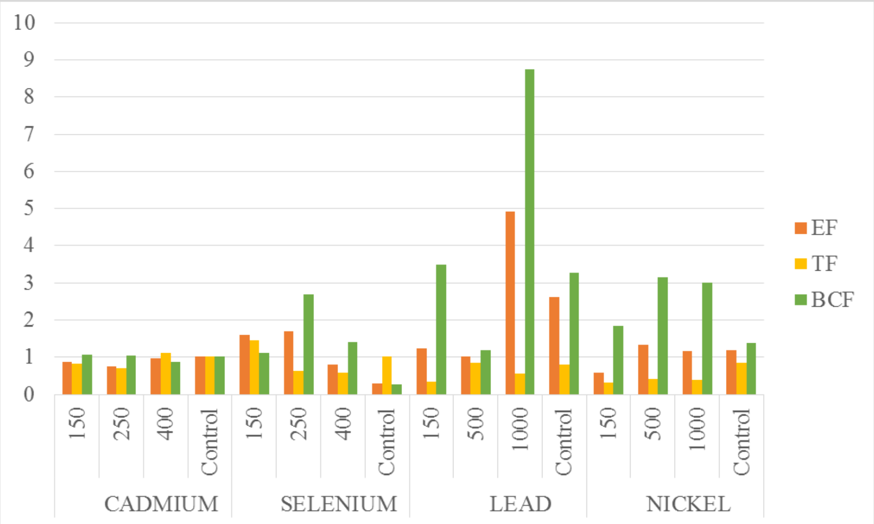

Figure 8: the Enrichment, Translocation, and Bioaccumulation Factors for Cd, Se, Pb and Ni.

CONCLUSION

Phytoremediation involves diverse use of plants for *in situ* treatment of metal contaminated soils, sediments, water and air. In this research work, the possibility of cleaning or decontaminating the environment of metal contamination using the grass *H. vulgare* was assessed. The result showed that, the plant, *H. vulgare* can best be defined and used as a stabilizer for Ni, Pb and Se in the soil. A process best described as phytostabilization. The non-toxic effect of Cd on the plant, despite the high concentration absorbed and translocated to the shoot and having the TF value greater than one, *H. vulgare* may serve as a phytoextractor and possibly hyperaccumulator when the concentration of the metal, Cd in the soil is considerably high and available for plant uptake.

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