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ORIGINAL RESEARCH ARTICLE

Phytoremediation Potential of *Ixora coccinea* in Multi-Metal Contaminated Soil

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ABSTRACT

This study examined the phytoremediation potential of Ixora coccinea through a greenhouse pot experiment. The plants were transplanted into 3 kg of soil artificially contaminated with Ni (as $NiSO_4 \cdot 6H_2O$), Pb (as $Pb(NO_3)_2$), and Co (as $CoCl_2 \cdot 6H_2O$) at concentrations of 1000 mg, 2000 mg, and 3000 mg, respectively. A separate pot with uncontaminated soil served as a control. The plants were watered with 650 mL of water every two days in the evening for sixteen weeks. At the end of the experiment, plant and soil samples were collected and carefully separated into roots and shoots. These components, along with the soil, were dried, ground, and sieved. The sieved soil, roots, and shoots from both the experimental and control groups were digested with aqua regia, followed by analysis using an Atomic Absorption Spectrometer (AAS). Bio-concentration Factor (BCF) and Translocation Factor (TF) were calculated for Co, Ni, and Pb. The BCF values of I. coccinea for Co, Ni, and Pb were 15.64, 1.32, and 1.50, respectively. The TF values for Co and Pb were below unity, while Ni showed the highest TF value of 2.3. These findings suggest that I. coccinea is a promising candidate for the phyto-extraction of Ni, as it exhibited both BCF and TF values greater than unity. However, its lower TF values for Co and Pb indicate a decreased affinity for these metals.

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INTRODUCTION

Heavy metals are a group of elements with atomic numbers exceeding 20 or specific gravities above 5 g/cm^3 (Durumin Iya *et al.*, 2022). These elements are naturally found in the Earth's crust and cannot be degraded, making them persistent environmental pollutants. They accumulate in soil through various processes (DesMarais and Costa, 2019; Luo *et al.*, 2020; Waleed and Hamad, 2023), and contamination arising from human activities has become a serious global environmental issue with profound effects on agricultural productivity, ecosystem health, and human well-being (Adamu, 2019; Bench, 2020; Esther *et al.*, 2023; Grzegórska *et al.*, 2023). The accumulation of heavy metals in soil threatens human health because they can enter the body via food consumption, with toxicity depending on concentration and duration of exposure (Grzegórska *et al.*, 2023). The Agency for Toxic Substances and Disease Registry (2012) and the United States Environmental Protection Agency (2019) classify metals such as As, Pb, Cd, Ni, and Hg among the top 20 most toxic and carcinogenic substances.

Several remediation techniques have been developed to restore contaminated soils, but most are costly and environmentally destructive (Asma *et al.*, 2019; Shah and Daverey, 2020; Shazia *et al.*, 2022). Phytoremediation, a plant-based approach, is gaining popularity due to its cost-effectiveness, environmental friendliness, and efficiency (Abdulazeez, 2017; Karishma *et al.*, 2018; Salisu and Ibrahim, 2024). Successful phytoremediation requires plants with dense and deep root systems, high biomass production, the capacity to thrive in heavily contaminated environments, and the ability to remediate multiple pollutants simultaneously. They should be native to their habitats, resistant to pests and diseases, less appealing to herbivores to reduce contaminant transfer, and physiologically adaptable to diverse environmental conditions (Rajput *et al.*, 2020).

Certain plant species have evolved to tolerate and even accumulate heavy metals in concentrations exceeding soil levels (Azhar *et al.*, 2022; Li *et al.*, 2022a; Musa *et al.*, 2023; Salisu and Ibrahim, 2024). Their tolerance mechanisms include restricting metal uptake, detoxifying absorbed metals, or excreting them (Onyia *et al.*, 2021). Based on this, plants are categorized as excluders, accumulators or hyperaccumulators, and indicators. Excluders restrict uptake into roots or translocation to shoots (Azhar *et al.*, 2022; Li *et al.*, 2022a). Accumulators and hyperaccumulators absorb and store high concentrations without toxicity symptoms, while indicators accumulate

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metals proportionally to soil concentrations, making them useful bioindicators (Durumin Iya *et al.*, 2019; Luo *et al.*, 2021; Mahdavian *et al.*, 2022; Adamu *et al.*, 2023; Musa *et al.*, 2023). Phytoremediation effectiveness is often measured using the bioconcentration factor (BCF) and translocation factor (TF), where values greater than one indicate suitability for phytoextraction (Li *et al.*, 2022a; Musa *et al.*, 2023). Although genetic engineering can enhance accumulation capacities (Asma *et al.*, 2019), native species remain preferable due to their adaptability and low maintenance requirements (Muhammad *et al.*, 2020).

While phytoremediation improves soil quality by removing heavy metals and preventing erosion (Aftab *et al.*, 2021; Kwoczynski and Čmelík, 2021; Durumin Iya *et al.*, 2022; Aqib *et al.*, 2023), improper disposal of contaminated biomass risks reintroducing metals into the environment (Khan *et al.*, 2021). To address this, post-remediation strategies include composting to generate nutrient-rich material (Aftab *et al.*, 2021; Kwoczynski and Čmelík, 2021; Aqib *et al.*, 2023), agromining and phytomining to recover valuable metals such as Au, Ag, and rare earth elements (Tisserand *et al.*, 2021; Aqib *et al.*, 2023), and thermochemical methods such as gasification and pyrolysis to convert biomass into biochar, syngas, and hydrogen (Nugroho *et al.*, 2021; Senthil and Lee, 2021). Biochar from contaminated biomass can also serve as a secondary adsorbent for metal removal (Yousaf *et al.*, 2022; Aqib *et al.*, 2023).

Human exposure to heavy metals remains unavoidable due to their ubiquity in air, soil, water, and food (Durumin Iya *et al.*, 2019; Nolos *et al.*, 2022). While some metals are essential micronutrients, excessive intake is toxic (DesMarais and Costa, 2019; Waleed & Hamad, 2023). Toxicity manifests through gastrointestinal disturbances, neurological disorders, respiratory impairment, and organ damage (Sanaei *et al.*, 2021; Nolos *et al.*, 2022). Mechanistically, toxicity results from reactive oxygen species generation and oxidative stress, enzyme inhibition and antioxidant disruption, and DNA damage leading to mutagenesis and carcinogenesis (DesMarais and Costa, 2019; Luo *et al.*, 2020; Ohiagu *et al.*, 2022). Chronic exposure also affects endocrine and reproductive function, contributes to neurodegenerative diseases such as Alzheimer's and Parkinson's, and impairs cognitive processes (Charkiewicz *et al.*, 2022; Kosare *et al.*, 2023; Luo *et al.*, 2020).

Within this context, *Ixora coccinea*, commonly known as flame of the woods or jungle flame, presents as a potential phytoremediator. This evergreen shrub of the Rubiaceae family, native to Southeast Asia, grows 1.2–1.8 meters high with dense branching, leathery oblong leaves, and clusters of small tubular flowers in red, yellow, pink, or orange. The novelty of this research lies in the discovery of *I. coccinea's* ability to thrive in heavy metal-contaminated soils, extract and accumulate metals, and translocate them to above-ground tissues under greenhouse conditions. To our knowledge, no previous study has evaluated the tropical ornamental shrub I. coccinea for simultaneous Co, Ni, and Pb removal under controlled greenhouse conditions.

MATERIALS AND METHODS

Sampling Area

The plant samples were gathered from Federal University Dutse, located at coordinates 11° 45' 22.25" N and 9° 20' 20.26" E, along Ibrahim Aliyu Bypass in Dutse LGA, Jigawa State, Nigeria. The soil supporting the plant growth was collected from a greenhouse at the Old Secretariat, situated at the same geographical coordinates (11° 45' 22.25" N, 9° 20' 20.26" E) along Government House Road, Dutse. The soil samples were collected by scraping off the surface layer to eliminate plants and debris before using a soil auger to extract samples from a depth of 15 cm. afterward, the soil samples were made into 2 portions and placed in a polyethylene flexible container. Half of the soil sample was used in the determination of physical and chemical parameters of the soil. The other half was air-dried, sieved through 2.00 mm pore size mesh in the laboratory and spiked with the salt of Co, Ni and Pb, and used for phytoremediation purposes.

Experimental Design

A greenhouse pot experiment was conducted for 16 weeks using 3 kg of soil per pot, following the method outlined by Adamu (2019) at the Old Secretariat in Dutse. The soil samples were spiked with the following heavy metals: Ni as $NiSO_4.6H_2O$, Pb as $Pb(NO_3)_2$, and Co as $CoCl_2.6H_2O$ at concentrations of 1000, 2000, and 3000 mg for Ni, Pb, and Co, respectively. The soil in each pot was thoroughly mixed, irrigated, and then the plants were transplanted. A separate pot containing untreated soil was used as a control. The experiments were exposed to natural day and night temperatures, as humidity is an essential factor for plant growth and physiological functions. Irrigation of the pots was carried out with 650 mL of water every two days in the evening. Plastic trays were placed under each pot to collect leachate, which was then returned to the pots to prevent the loss of nutrients and metals from the samples (Garba et al., 2017). Three replicates of each experimental pot were prepared for statistical analysis.

Sample Processing

Upon completion of the experimental phase, the plants were removed from their pots and meticulously rinsed in the laboratory using distilled water. They were then carefully divided into roots and shoots. Each part was air-dried at room temperature until a consistent weight was achieved. Subsequently, the dried plant material was ground and sifted through a 2.00 (pore size) mm mesh screen for further analysis., as described by Razzaq (2017). The soil samples were also collected, homogenized, and dried at 70°C to a constant weight, ground, and then sieved through a 2 mm mesh. The sieved soil, along with the shoots and roots of the plants, were stored in plastic sample bottles for the next analysis, as stated by Khan et al. (2018).

Digestion of soil and plant tissue samples

Each plant tissue (3.0 g) was separately digested with 20 mL of concentrated HCl + HNO_3 (3:1 V/V) on a hot plate in a fume cupboard until white fumes were produced. The heated solution was then allowed to cool to room temperature, filtered through Whatman No. 42 filter paper into a 50 mL standard volumetric flask, and made up to the mark with deionized water (Adamu, 2019). Each soil sample (3 grams) was treated with 20.0 mL of a $HNO_3 + HClO_4$ (5:1 V/V) mixture and heated to 95°C ± 5°C on a hot plate in a fume cupboard until white fumes were observed. The solution was then cooled to room temperature, filtered through Whatman No. 42 filter paper into a 50 mL standard volumetric flask, and made up to the mark with deionized water (Razzaq, 2017). The samples were analyzed with Atomic Absorption Spectroscopy (AAS) at Bayero Universty Kano.

Heavy metals Analysis using AAS

Determination of heavy metals in the samples were performed on a Perkin Elmer Atomic Absorption Spectrophotometer (AAS) Model Optima 8300 series using acetylene, nitrous oxide and compressed air for burning. The gases have a specific flow rate based on the element of interest. Flame atomiser is divided into two, total consumption burner and premixed burner. Temperature of the rod is raised to dry and atomise the sample in a chamber. A hollow-cathode lamp (HCL) are made of the metal of the substance to be analyzed. The anode is made of Tungsten with each HCL has a particular current for optimum performance. High currents produced brighter emission and less baseline noise. Deuterium lamp was used to calibrate the wavelength for background purposes. Standard solutions were prepared by using 1% HNO_3 and deionized water. Three different concentrations were prepared by adding suitable volume of stock standard solution (100 mg/kg) to 100 mL capacity volumetric flask and top up with deionized water to the mark.

Validation of Atomic Absorption Spectrophotometer Data

Several parameters have been used to validate the method for determination of heavy metals using AAS by analyzing blank sample and standard samples with a series of heavy metals concentrations. Calibration graphs of absorbance versus concentration for each heavy metals were then plotted. Limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified, under the stated conditions of the test (Topic et al., 2015). LOD can be estimated using the equation 1 below.

$$\text{LOD}=3^{*}\ (\text{S.D blank}/m) \qquad \text{Equation 1}$$

where S.D blank is standard deviation for 10 replicates analysis of blank sample and m is sensitivity that can be obtained from a slope of calibration graph. Limit of quantitation (LOQ) is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test (Shrivastava & Gupta, 2011).

$$\text{LOQ}=10^{*}(\text{S.D blank}/m) \qquad \text{Equation 2}$$

Limit of linearity (LOL) is defined as the range of concentrations that an instrument gives a linear response (Carlson et al., 2014). A steeper line with a large slope indicates a more sensitive instrument. Limit of linearity is denoted by C from calibration curve equation. Dynamic range is defined as the range of lower and higher values between LOQ and LOL.

$$\text{Dynamic range} = \text{LOQ to LOL} \qquad \text{Equation 3}$$

Statistical Data Analysis

The reported data represent averaged values, along with the standard deviation (SD) and standard error, derived from five reliable replicates chosen from eight independent replicates. Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA). Variations in concentration across different harvesting times were deemed statistically significant at a p-value of less than 0.05. The statistical analysis was conducted using IBM SPSS Statistics version 24. To evaluate the plant's potential for phyto-extraction and phyto-stabilisation of heavy metals, two indices were utilized: the Bio-concentration Factor (BCF), and Translocation Factor (TF). These were calculated using equations 1 and 2, respectively (Durumin Iya et al., 2018). Plants are considered suitable for phyto-extraction if they exhibit BCF values exceeding 1. Conversely, they are ideal for phyto-stabilisation if they demonstrate high BCF values combined with low TF values (Durumin Iya et al., 2018).

The ability of *I. coccinea* to function as a phytoextractor or Phyto stabilizer of heavy metals was evaluated using two key indices: the bio-concentration factor (BCF) and the translocation factor (TF). The BCF is calculated as the ratio of metal concentration in the plant's entire tissue to the initial metal concentration in the soil, while the TF is determined by the ratio of metal accumulation in the shoots to that in the roots.

Quality Control

Stringent safety protocols and quality control measures were implemented to guarantee the accuracy and dependability of the findings. To prevent cross-contamination, samples were meticulously managed. Glassware underwent a rigorous cleaning process, which included washing with detergent, thorough rinsing with deionised water, soaking in a 25% nitric acid solution, and a final rinse with deionized water before being dried in an oven. All reagents employed were of analytical grade, and every piece of equipment was calibrated in accordance with the manufacturer's instructions before use.

RESULTS AND DICUSSION

Physicochemical Analysis

Table 1.0 presents the measured physicochemical properties of the experimental soil. The soil was classified as loamy sand, with its composition consisting of 7.08% clay, 71.09% sand, and 77.17% silt. The pH and EC values were 6.57 and 208.3 µS/cm. The nature of the soil is less acidic which plays a vital role in the metal bioavailability and proper uptake of heavy metals by plant from soil, this is in agreement with the report of Kanwar et al. (2020). The concentration of nitrogen and phosphorus found were 0.43% and 5.16 mg/kg, respectively. Phosphorus is a key component of several compounds involved in photosynthesis and respiration within plants, while nitrogen is crucial for energy metabolism and protein synthesis, both of which are vital for plant growth and development (Nwakife et al., 2022). The organic matter (OM) content was found to be quite low at 0.61%, and the cation exchange capacity (CEC) was also low, recorded at 9.31 Cmol/100 Kg. According to Shah and Daverey (2020), OM plays a significant role in the physical and chemical fertility of soil, while CEC reflects the soil's ability to retain exchangeable cations and serves as an indicator of its nutrient storage capacity. The low concentrations of clay and CEC suggest an increased mobility of heavy metals in the soil, which can enhance the uptake of these metals by plants. This finding aligns with the observations of Atafar et al. (2010).

The control soil (CS) was analyzed to represent the background value of the soil at its natural state. The data obtained were found to be 6.23 ± 0.02, 7.39 ± 0.01, 4.09 ± 0.03 mg/kg for Co, Ni, Pb respectively. And these results are within the recommended permissible limit as ascertained by Joseph et al. (2020); Charkiewicz et al. (2022) who described that the recommended level of natural nickel content in soil range from 0.2 to 450 mg/kg, levels of lead in soil range between 50 to 400 mg/kg and level of cobalt in soil range between 0.1 to 70 mg/kg respectively.

Table 1: Physicochemical Properties of the Experimental Soil

Parameters	Results
pH	6.57 ± 0.09
Electrical conductivity (µS/cm)	208.3 ± 0.13
CEC (Cmol + kg^{-1})	19.31 ± 0.11
Organic Carbon (%)	0.39 ± 0.42
Organic Matter (%)	0.61 ± 0.94
Nitrogen (%)	0.43 ± 0.01
Available P (mg/Kg Soil)	5.16 ± 0.02
Ca^{2+} (Cmol (+)/100 kg Soil)	4.37 ± 0.04
Mg^{2+} (Cmol (+)/100 kg Soil)	7.62 ± 0.01
Co^{2+} (mg kg^{-1})	6.23 ± 0.02
Ni^{2+} (mg·kg^{-1})	7.39 ± 0.01
Pb^{2+} (mg·kg^{-1})	4.09 ± 0.03
Sand (%)	71.09± 0.02
Silt (%)	77.17± 0.01
Clay (%)	07.08 ± 0.01
Soil Texture Class	Sandy loamy

Table 2: Parameters obtained from method validation for 3 heavy metals using AAS

Heavy metals	LOL (mg/kg)	LOQ (mg/kg)	LOD (mg/kg)
Co	4.93	0.36	0.16
Ni	4.73	0.31	0.12
Pb	4.79	1.04	0.19

Table 3: concentration (mg/kg) of Co in contaminated soil, root and shoot of the plant

	4th week			8th week			12th week			16th week		
	soil	root	shoot	soil	root	shoot	soil	root	shoot	soil	root	shoot
1000	4.04±0.37	8.33±1.22	5.48±0.82	3.28±2.03	15.04±1.01	9.17±0.77	2.53±0,13	21.44±0.17	13.09±1,04	1.41±0.03	29.66±1.10	18.79±1.22
Control	2.15±0.01	0.51± 0.02	0.29± 0.01	1.83±0.01	0.74±0.01	0.53±0.00	1.35±0.01	1.11±0.02	0.80±0.03	0.71±0.03	1.12±0.03	0.91±0.02
2000	6.99±0.44	17.29±1.00	13.42±1.05	5.09±1.51	30.11±0.25	21.38±0.31	3.98±0.18	42.04±0.99	26.57±1.01	2.36±0,23	67.34±1.98	32.71±1.66
Control	2.23±0.02	0.50± 0.02	0.31± 0.01	1.81±0.01	0.74±0.01	0.53±0.00	1.36±0.01	1.02±0.02	0.83±0.01	0.73±0.03	1.11±0.03	0.93±0.02
3000	9.47±0.08	28.65±1.27	17.14±0.98	7.75±0.19	59.72±1.33	33.26±1.00	6.22±0.82	81.31±1.92	45.38±1.22	4.47±0.08	103.1±0.04	69.92±0.02
Control	2.18±0.02	0.52± 0.02	0.30± 0.01	1.83±0.01	0.74±0.01	0.54±0.00	1.36±0.01	1.04±0.02	0.81±0.01	0.73±0.03	1.11±0.03	0.94±0.02

Data are presented as Mean ± SD (n=3). No significant difference was observed at P < 0.05 using ANOVA Analysis and Multiple Comparison according to Tukey Test, SD = Standard Deviation

Table 4: concentration (mg/kg) of Ni in contaminated soil, root and shoot of the plant

	4th week			8th week			12th week			16th week		
	soil	root	shoot	soil	root	shoot	soil	root	shoot	soil	root	shoot
1000	15.48±0.86	8.94±1.32	10.40±0.26	12.37±1.81	12.11±1.94	15.66±1.77	9.33±1.2.01	15.02±1.40	19.28±2.05	5.64±0.15	18.49±0.23	24.02±0.91
Control	7.41 ± 0.01	1.12 ± 0.01	1.23 ± 0.06	6.90 ± 0.01	1.40 ± 0.01	1.63 ± 0.06	5.11 ± 0.02	1.68 ± 0.01	1.92 ± 0.03	7.40±0.01	1.10 ± 0.01	1.20 ± 0.06
2000	18.95±1.36	11.87±1.10	18.15±1.22	16.82±0.99	14.11±1.00	22.49±1.08	12.13±0.34	17.48±0.18	26.89±0.22	8.28±0.39	21.09±0.32	29.65±0.46
Control	7.39 ± 0.01	1.09 ± 0.01	1.31 ± 0.03	6.87 ± 0.01	1.41 ± 0.02	1.65 ± 0.03	5.23 ± 0.01	1.71 ± 0.02	1.94 ± 0.01	7.28±0.02	1.99 ± 0.02	2.20 ± 0.06
3000	24.17±2.19	19.63±2.11	26.92±0.98	20.74±0.55	23.46±0.18	30.08±1.04	16.85±0.44	28.61±0.91	32.48±1.02	9.39±0.04	32.55±0.37	33.05±1.06
Control	7.37 ± 0.01	1.09 ± 0.01	1.32 ± 0.02	6.85 ± 0.02	1.39 ± 0.02	1.63 ± 0.01	5.24 ± 0.02	1.69 ± 0.02	1.92 ± 0.01	7.09±0.02	1.95 ± 0.01	2.13 ± 0.02

Data are presented as Mean ± SD (n=3). No significant difference was observed at P < 0.05 using ANOVA Analysis and Multiple Comparison according to Tukey Test, SD = Standard Deviation

Table 5: concentration (mg/kg) of Pb in contaminated soil, root and shoot of the plant

	4th week			8th week			12th week			16th week		
	soil	root	shoot	soil	root	shoot	soil	root	shoot	soil	root	shoot
1000	22.05±1.33	14.33±0.18	11.68±0.29	14.01±0.83	19.61±1.04	16.72±0.22	11.39±0.09	25.69±0.48	20.82±0.37	7.03±0.66	27.11±0.82	23.51±0.77
Control	2.93±0.03	0.26±0.01	0.31±0.01	2.56±0.03	0.34±0.03	0.38±0.01	2.09±0.00	0.42±0.03	0.46±0.02	1.68±0.00	0.57±0.01	0.61±0.03
2000	26.99±2.01	28.91±0.22	26.44±0.09	22.48±1.01	36.08±0.77	32.45±0.36	16.09±0.51	48.09±0.90	38.57±1.11	8.44±0.03	59.53±0.84	42.07±1.34
Control	3.43±0.05	0.33±0.08	0.37±0.06	3.19±0.06	0.51±0.09	0.58±0.03	2.73±0.03	0.63±0.04	0.68±0.05	2.31±0.06	0.87±0.05	1.06±0.04
3000	34.78±2.11	30.33±1.07	25.38±1.63	28.33±0.72	37.58±0.18	31.44±0.93	22.09±1.26	48.25±1.03	36.08±1.38	14.37±0.08	56.98±0.55	43.63±0,91
Control	3.98±0.03	0.38±0.01	0.42±0.02	3.61±0.01	0.58±0.04	0.65±0.03	3.19±0.03	0.64±0.01	0.79±0.00	2.83±0.02	0.68±0.03	0.87±0.01

Data are presented as Mean ± SD (n=3). No significant difference was observed at P < 0.05 using ANOVA Analysis and Multiple Comparison according to Tukey Test, SD = Standard Deviation

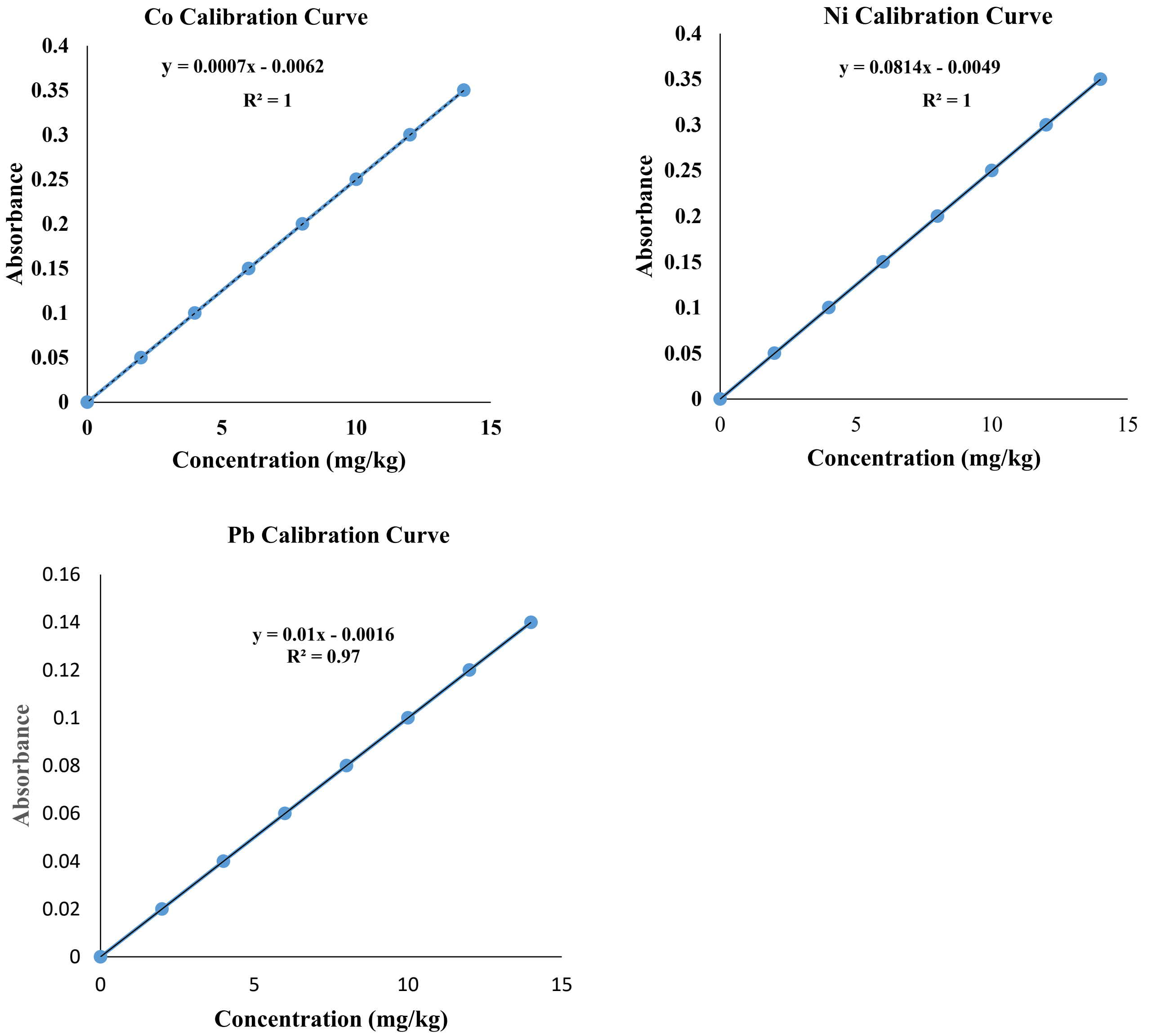

Figure 1: The calibration graph of Co, Ni and Pb (where standard solutions of the metals were used in Atomic Absorption spectrophotometer, and the AAS absorbance was plotted against concentration)

Growth Tolerance of *I. coccinea* Plants

On harvesting period of 4th, 8th, 12th, and 16th weeks, *I. coccinea* plants were removed gently from contaminated and control soil. The root lengths of the plants grown on Co, Ni and Pb treated soil and control soil were measured and recorded as 27.06 – 28.05 cm, 20.11 – 29.09 cm, 12.31 –19.10 cm, and 20.02 – 22.61 cm respectively. Whereas shoot lengths of the plants for Pb, Ni, Co contaminated soil and control soil varied between 10.06 –11.13 cm 12.50 – 18.21 cm, 13.07 –14.10 cm, and 18.01 – 22.61 cm respectively. Plants attained about 93 % overall growth in the treated soils when compared to the growth in untreated soil. While the plant attained a total above ground (shoots and leaves) dry weight grown on contaminated soil per experimental pot with about 85% compared to that grown on control soil. It can be perceived that *I. coccinea* plants can demonstrate better growth potential when planted on contaminated soil as compared to that on uncontaminated soil (control soil).

Calibration graphs and method validation parameters

Calibration curves of heavy metals from standard solution are presented on Figure 1 for (Co, Ni and Pb). Parameters such as, LOL, LOQ, LOD, sensitivity, dynamic range and regression (R^2), were obtained from method validation of 3 heavy metals using AAS and presented on Table 2.

Validation of heavy metals by AAS

Table 2 shows the parameters from the method validation for three (3) heavy metals determined by the use of AAS. The parameters obtained were LOL, LOQ and LOD (see Table 2).

Decrease in soil metal levels

Upon completion of the phytoremediation study, a significant decline in metal concentrations was observed across all soil samples.

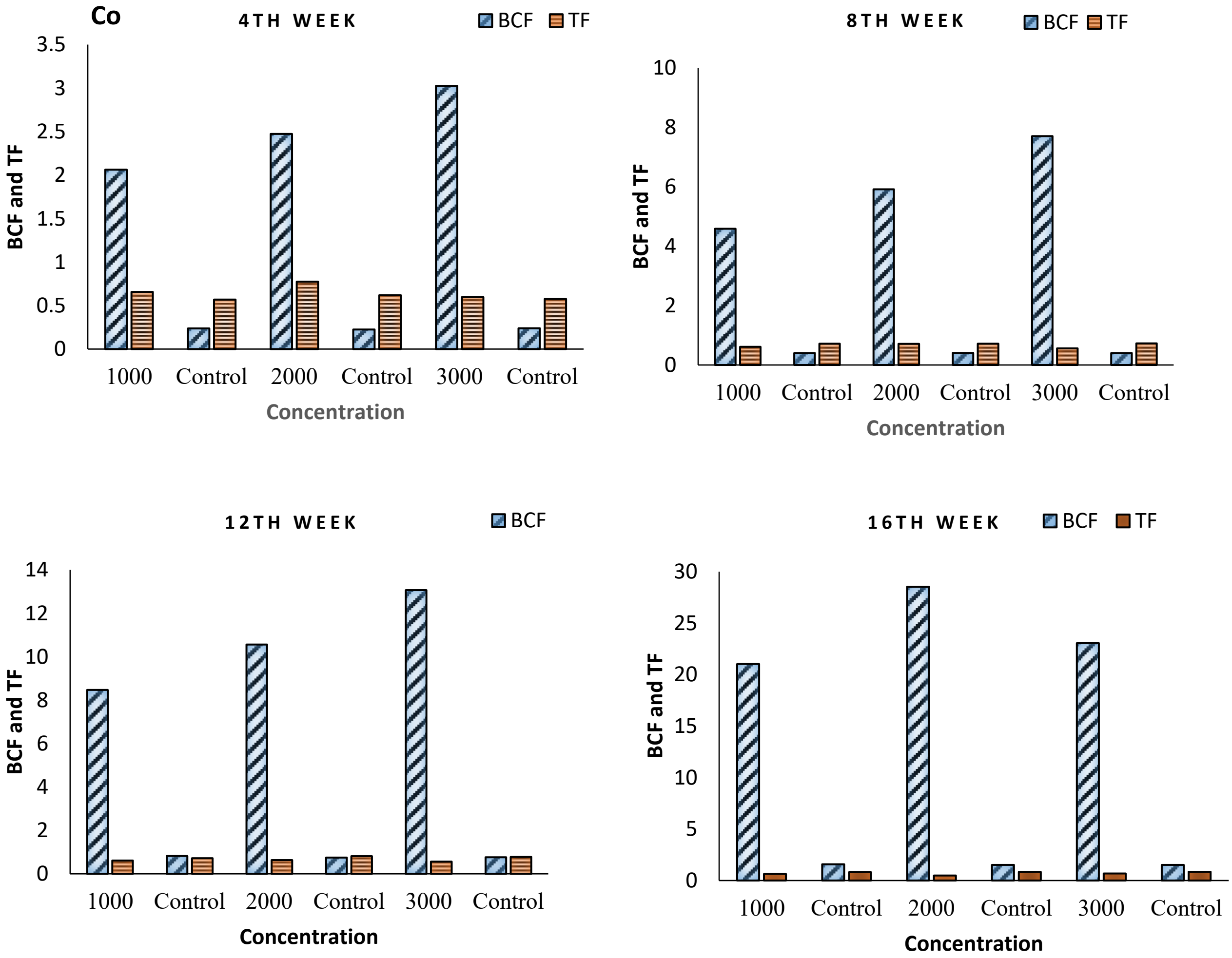

Figure 2: The graph of BCF and TF for Cobalt plotted against concentration

The reductions were recorded at 96.99% for cobalt (Co), 64.59% for nickel (Ni), and 78.86% for lead (Pb). In comparison, the control soil exhibited much lower reductions of 21.34% for Co, 24.27% for Ni, and 17.58% for Pb. These findings highlight the effectiveness of plants in lowering metal content in contaminated soils. The stark contrast between the treated and control soils underscores the ability of phytoremediation to significantly mitigate heavy metal concentrations.

Heavy Metals Uptake by Plant

The uptake of heavy metals (Co, Ni, and Pb) in the root and shoot tissues of plant was investigated. Table 2, 3 and 4 shows the accumulation and translocation of Co, Ni and Pb respectively. Heavy metals uptake in different plant parts (root and shoot) harvested from contaminated and control soil on 4th, 8th, 12th and 16th weeks was presented. A significant ($p < 0.05$) quantities of heavy metals were observed in the root more than the shoot except for Ni which was accumulated more in the shoot on all the harvesting period. In this experiment, heavy metals uptake by the roots were in the ranges of 8.33-103.11mg/kg Co. Moreover, there was no accumulation of heavy metals in the plant grown on untreated soil (control soil) as it was expected because the control soil does not contain heavy metals. Plants can selectively absorb various elements. They are capable of taking up heavy metals not only through their roots but also via stems and leaves, subsequently sequestering them within their tissues (Kanwar et al., 2020).

Uptake and Accumulation of Cobalt

Table 2.0 shows the results of cobalt accumulated in the root and translocated to the shoot of the plant on different harvesting period. The uptake and accumulation of Co by *I. coccinea* plant was found to be higher in the root (103.10 mg/kg) grown on 3000 mg/kg contaminated soil on 16th week harvesting period compared to the shoot (69.92 mg/kg).

Other accumulation and translocation of Co has occurred in the plants grown on 1000 and 2000 mg cobalt contaminated soils with increasing concentration from 4th to 16th weeks as shown in Table 2. Studies show that **cobalt (Co)** tends to accumulate preferentially in roots, with only a small proportion being transported to stems and leaves due to low xylem loading and strong vacuolar sequestration in root cells. Consequently, when root Co levels remain significantly higher than those in aerial tissues, it is a clear indicator of poor translocation efficiency (Azhar et al., 2022; Li et al., 2022) and the inability of Co^{2+} ions to traverse key physiological barriers—namely, the plasma membrane at the cellular level and the endodermal layer at the tissue level—leads to their sequestration within cellular vacuoles. Plants

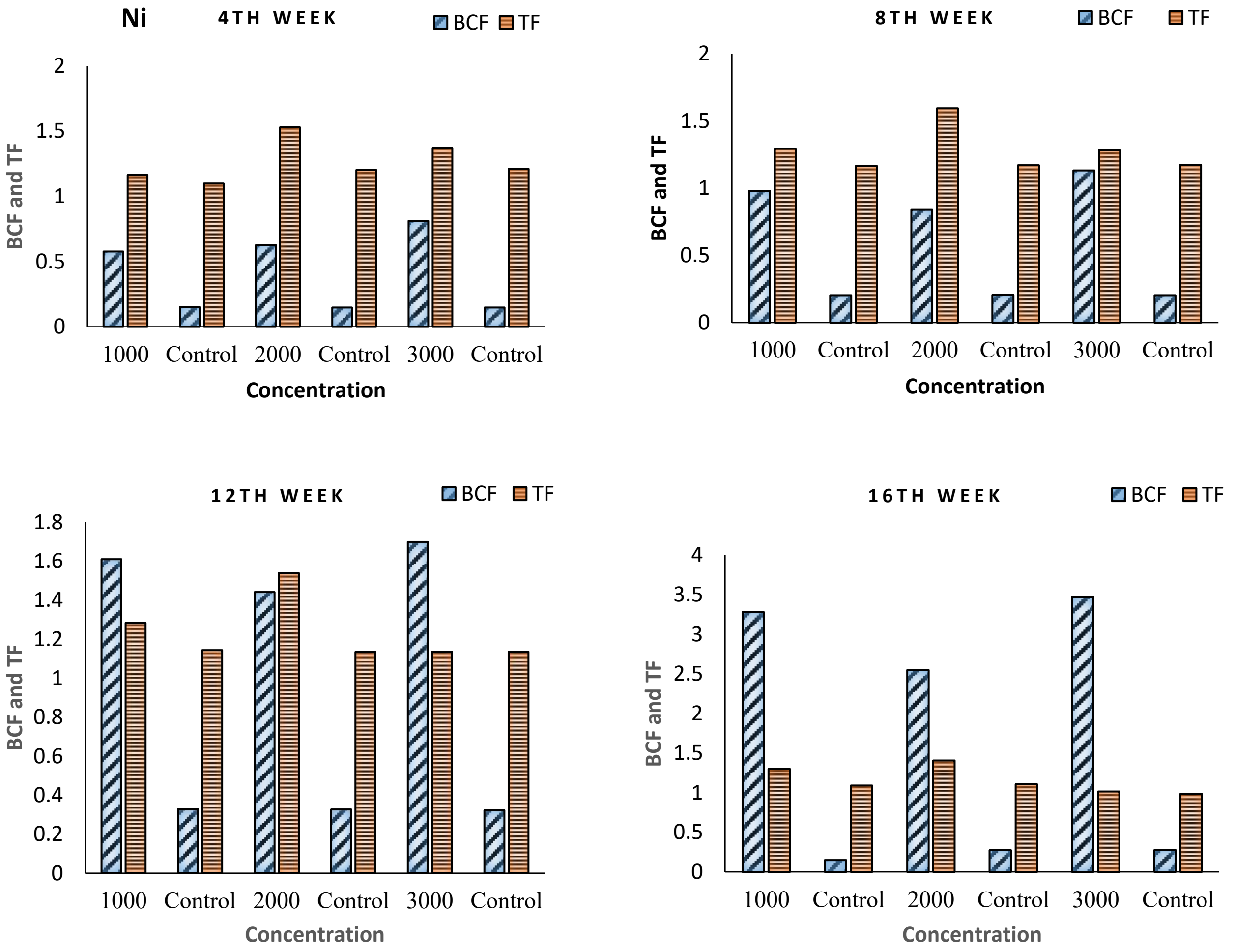

Figure 3: The graph of BCF and TF for Nickel plotted against concentration

employ multiple strategies to limit Co mobility by restricting its passage across membranes. This could be due to Co ions having difficulty traversing the hydrophobic core of the lipid bilayer that forms the plasma membrane (Azhar et al., 2022). The result is in accordance with the report of Gajić et al., (2018) who stated that some plants called excluders, are capable of reducing the uptake of pollutants inside their tissue over a wide range of soil concentration, i.e. they immobilize pollutants in their roots. Therefore, *I. coccinea* plant species seems to have lower affinity for Co phyto-extraction.

Uptake and Accumulation of Ni

Nickel is a vital nutrient for plants, although the quantity needed for their normal growth is minimal. Rapid industrialization and urbanization have caused the level of Ni in the environment to increase, thus, having a high potential to enter the food chain. Therefore, the uptake of Ni by plants is related to its toxicity, which may have possible implications with respect to humans and animals through the food chain (Alice et al., 2023). Several studies have reported that elevated concentrations of nickel in the nutrient medium can stimulate growth in higher plants, leading to increased biomass production without any noticeable signs of toxicity (Yuanita et al., 2021).

Under experimental conditions, plants cultivated in soil amended with 1000 mg Ni kg^{-1} exhibited superior biomass production and substantial nickel accumulation—with no visible phytotoxic effects—compared to those grown in soils treated with 2000 and 3000 mg Ni kg^{-1} (Table 3.0). Notably, Ni uptake was significantly elevated in the shoot tissues. The findings revealed that Ni concentration absorbed by plant was found to be higher in the shoot with mean value of 33.05 mg/kg and 32.55 mg/kg on the 16th week of harvesting period. It was observed that, no significant difference that exist between the concentration of Ni in the root and shoot of the plant on the 16th week harvesting period. This result concord with the observations of Pooja et al., (2020) who reported high level of Ni in the shoot (26.56 mg/kg) of *Rumex dentatus* plant than in the root (21.54 mg/kg). This could be due to the excess Ni which affected the biochemical process of the plant causing impairment of the membrane permeability which is associated with enhanced extracellular peroxidase activity and reduced the translocation of Ni from root to shoot (Yuanita et al., 2021). However, the plant can be regarded as good phyto-extractors of Ni. The results were found not statistically different at ($p < 0.05$).

Uptake and Accumulation of Lead

The concentration of Pb in plant was found to be highest in root with an average mean value of 59.53 mg/kg from

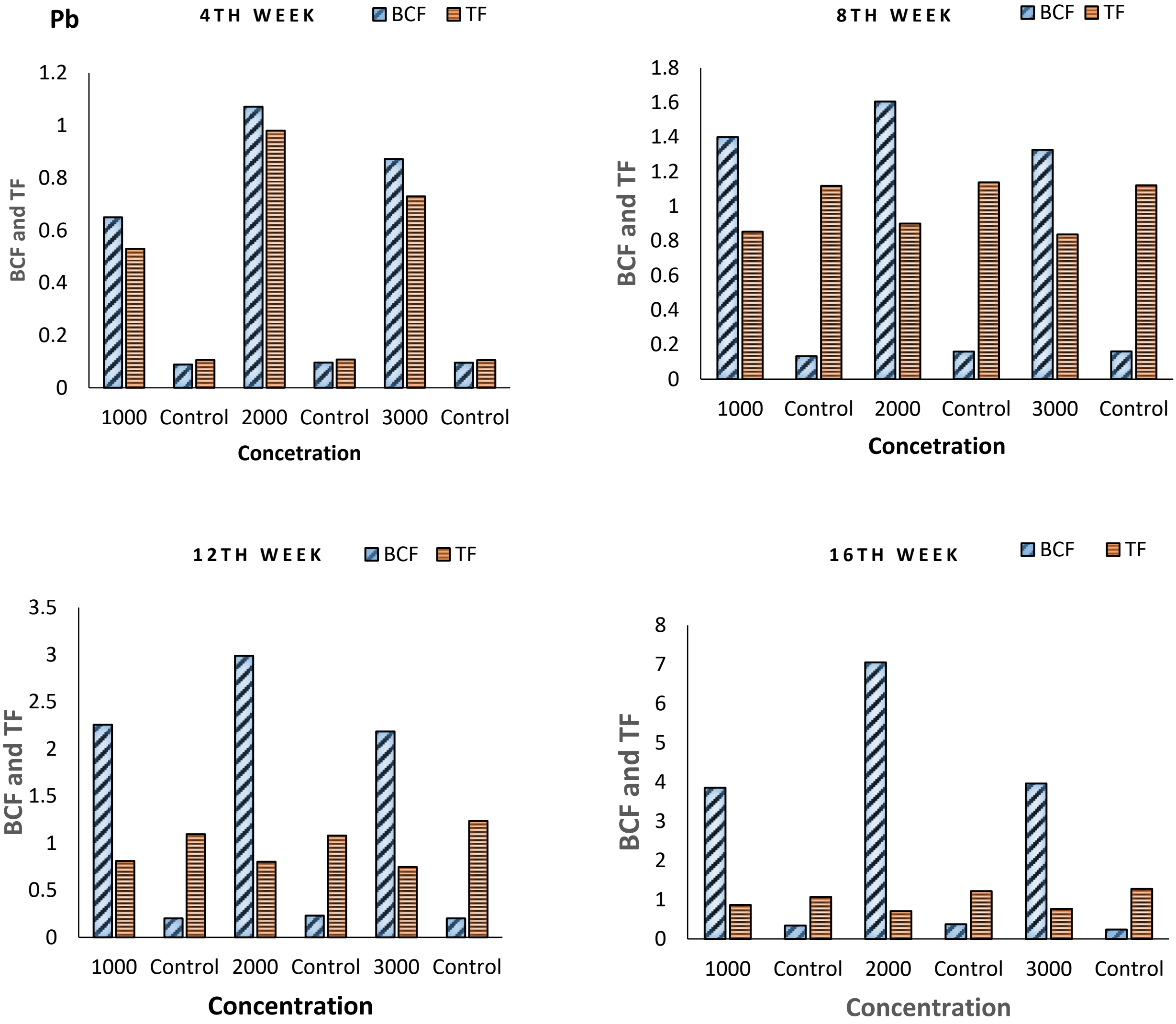

Figure 4: The graph of BCF and TF for Lead plotted against concentration

the plant grown on 2000 mg Pb contaminated soil compared to 43.63 mg/kg found in the shoot of 3000 mg Pb contaminated soil (Table 4.0). Similarly, in control sample the average concentration of Pb was also greater in the root of plant than in shoot as shown in Table 4.0. The results are in accordance with the report of Pooja et al., (2020), where they observed the accumulation of Pb in the root of *Ranunculus sceleratus* plant to be higher with mean value of 11.20 mg/kg compared the shoot with mean value of 9.21 mg/kg.

Plant potentiality in phytoremediation

The plants efficiency for phyto-extraction was evaluated using two parameters; Bio-concentration Factor (BCF) and Translocation Factor (TF). The BCF of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil. While, TF is an indicator of the translocation ability of metal from root to shoot. BCF and TF are necessary to measure a plant's potential for metal accumulation. If BCF and TF are more than unity (1), it indicates that the plants' phytoremediation effectiveness (Adamu, 2019).

Bio-concentration Factor (BCF)

The efficiency of bioaccumulation by the plants was assessed. All BCF values exceeded 1, indicating that the concentrations of Co, Ni, and Pb in the plant tissues were higher than those in the surrounding soil. For control soil, the BCF values of the three different metals were found to be less than unity, respectively. These suggest that the plant has low ability to absorb Co, Ni and Pb from the soil at low concentration.

The BCF values for the experimental conditions (1000, 2000, and 3000) are significantly higher than the Control values. The highest BCF value is observed at 3000 except for the 16th week in which the high value appeared 2000, and the Control values are consistently low. The TF values are more variable across conditions, and it's almost the same with the value of control. The Control TF values remain relatively stable

The BCF values for the experimental conditions (1000, 2000, and 3000) are significantly higher than the Control values. The BCF values were observed to be lower than the TF on 4th and 8th weeks, but higher than TF on 12th and 16th harvesting period. The Control TF values remain relatively stable at either 1 or above 1.

The BCF values for the experimental conditions (1000, 2000, and 3000) are significantly higher than the Control values. The highest BCF value is observed at 2000, while the Control values are consistently low. The TF values are more variable across conditions and were reducing gradually from 4th week to 16th week harvesting period.

Translocation Factor (TF)

The effectiveness of translocation by the plants were evaluated by TF. Translocation capability was evaluated from TF root to shoot as presented in Figure 4. The TF values in plant for spiked experiment were found to be 0.7, 2.3 and 0.6 for Co, Ni and Pb respectively. This data indicated that Co and Pb accumulated by the plant were largely retained in the root while Ni was effectively translocated into the shoot part of the plant. The result suggested that the plant can be used for the remediation of soil contaminated with Ni. This is in line with the view of Adamu et al., (2023) that if TF is greater than 1, the plant is suitable for phytoremediation of the target heavy metal. In contrast, the plant cannot be used for the phytoremediation of Co and Pb.

CONCLUSION

This research has shown a solar-powered, eco-friendly, and cost-effective way to remove metals from polluted soil by plants' inherent ability. This research evaluated the bioaccumulation and translocation of Co, Ni and Pb in *I. coccinea* Plants. Findings from this study have demonstrated the potential of *I. coccinea* plant in this research has higher BCF and TF of Ni which showed its suitability for Phyto-extraction of soil contaminated with Ni. In contrast, the TF values of Co and Pb were below unity. This indicates that Co and Pb were retained in the roots. Hence, the plant cannot be used for the remediation of soil contaminated with Co and Pb. Future studies could explore the mechanisms of heavy metal uptake, accumulation, and translocation in *I. coccinea* as well as its performance in field conditions and its potential for large-scale remediation projects.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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