Phytoremediation Potential of Cyperus Rotundus L. in Soils Contaminated with Potentially Toxic Elements, the Case of Abandoned Artisanal Small-Scale Gold Mines in Kenya

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ABSTRACT

Phytoremediation involves the use of plants for removal of pollutants from soil before its conversion for agricultural use. Cyperus rotundus L. is a reed that naturally grows in marshy and swampy areas including abandoned artisanal small-scale gold mines. This study was aimed at determining the potential of C. rotundus reeds for removal of Cu, Pb and Cd from soil polluted by artisanal small-scale gold mining (ASGM) activities in in western Kenya. C. rotundus plant and soil samples were collected in triplicate from five randomly selected localities where ASGM is practiced in western Kenya. The control samples were sourced from a locality where ASGM operations have never been conducted. After washing and separating the plants samples into leaves and roots, both soil and plant samples were air dried, oven dried and milled to fine powder. The replicate samples from each locality were combined to form a composite sample which was then digested and the levels of Cu, Pb and Cd, determined using an atomic absorption spectrophotometer in triplicate. Data analysis was done using Microsoft Excel version 2013. The levels of Pb, Cu and Cd, in the samples from the abandoned mines were higher than those of control samples. The levels of metals in the soil samples collected from the mines were in the order Cu>Pb>Cd. Quantities of the metals in the soil, root and leaf samples were in the order root>soil>leaf for Pb and Cd, while for Cu, it was roots>leaves>soil. The BCF values for C. rotundus roots and leaves of the different metals were in the order Cu>Pb>Cd and Cu>Cd>Pb respectively. The TF values for Cu (0.85±0.01 - 0.99±0.03) were highest followed Cd (0.42±0.03 - 0.86±0.03) and lowest for Pb (0.31±0.03 - 0.80±0.01). The MAI values were in the range 66.6-101. Uptake of Cu by the plant results in increased uptake of Pb but decreased uptake of Cd from the soil, while the uptake of Pb is not affected by availability of Cd in the soil and root tissues. Phytoremediation of Cu, Pb and Cd from soil polluted ASGM activities using C. rotundus is an environmentally friendly and cost-effective method of reduction of heavy metal toxicity in the soil.

Key words: Heavy metal toxicity, Bioconcentration factor, translocation factor, metal accumulation index

INTRODUCTION

Soil is an essential resource for natural living conditions of plants, animals and humans. Accumulation of excess metals in soil over an extended period exposes plants, humans and animals that depend on it to heavy metal toxicity [1]. Gold mine wastes have been reported to be among the major sources of pollutants which impact heavily on water and soil in numerous countries around the world including the United States, Mexico, China and South Africa among others [2]. For instance, in South Africa over 400 km^2 surface area of land within the Witwatersrand Basin is covered by about 300 unvegetated mine tailings and dumps. Mine tailings are the materials left over after separating the valuable fraction from the uneconomic fraction of an ore, while mine dumps are large mounds or hills of mining waste at the surface of a mine which contain waste rock or other material that overly an ore or mineral body and is displaced during mining without being processed.

Informal gold mining, also referred to as artisanal small-scale gold mining (ASGM) is mainly conducted using low technology or minimal machinery [3]. Over the years, small scale gold miners have operated globally without the environmental management strategies and control technologies that are considered standard practices among larger mining companies, contributing to environmental concerns over the proposed mines [4]. In Kenya, ASGM operations have been in practice since early 19th century and are concentrated mainly around Lake Victoria basin and some areas in Rift Valley [5]. These activities are mainly practiced in the three former provinces in Kenya including the Rift Valley (Turkana, West Pokot and Baringo counties), Nyanza (Migori, Siaya and Kisumu counties) and Western (Vihiga and Kakamega counties) provinces [5, 6]. In Kakamega and Vihiga counties, it is estimated that thousands of people are involved in ASGM activities which are mainly concentrated both inland and along streams and rivers, and are responsible for soil and water pollution resulting from mining wastes such as solid wastes tailings and liquid waste-effluents [5].

ASGM activities in Kenya were formalized and legalized by the promulgation of the new mining act in Kenya, “Mining Act No. 12 of 2016 (section 98, subsections 2 and 3)” which allows for licensing and putting in place a framework for best practices in a sector that was previously been considered illegal and informal [7]. However, the act does not provide specific regulations on the health and safety of ASGM activities.

The rapid expansion of ASGM practices globally over the years has resulted in increased health concerns involving toxic pollutants from the sector that pose major environmental hazards to both aquatic and terrestrial ecosystems, greatly altering the biochemical status of the soil [8, 9]. ASGM operations mainly include ore excavation, crushing, milling and gravity separation on sluices. These activities may result in the release of potentially toxic elements (PTEs) through mine effluents such as wastewaters, tailings, and runoff from mine dumps, which often pollute the surrounding soils and water bodies. Some of the PTEs associated with gold mining and processing include copper, nickel, mercury, zinc, silver, lead, titanium, arsenic, cadmium and chromium [7, 10, 11]. These metals have serious health implications including, diseases and ailments such a gastrointestinal and kidney dysfunction, nervous system disorders, skin lesions, vascular damage, immune system dysfunction, birth defects and cancer among others [12, 13, 14]. Human beings are exposed to the PTEs via direct ingestion, contact with contaminated soil, the food chain (soil–plant-human or soil–plant–animal-human) and drinking of contaminated ground water. ASGM activities also lead to a decrease in food quality via phytotoxicity and reduction in land usability for agricultural production causing food insecurity and land tenure problems.

Western Kenya is faced with countless environmental challenges including environmental degradation via continuous release into the environment of PTEs arising from ASGM activities among others [15, 16]. The mining activities are widely spread along rivers and streams traversing Kakamega and Vihiga counties [16]. After exhaustion of the mineral, the mines are abandoned without any cleanup of the PTEs and are mostly converted to agricultural activities including cultivation of crops and grazing animals, hence posing danger to plants, animals and humans.

Phytoremediation is one of the environmentally friendly and economic methods that are used for land rehabilitation. Land rehabilitation is the process of restoring land that has been destroyed by human activities to its former good condition. Phytoremediation involves the treatment of environmental problems by the use of plants that mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere [17]. These plants are used to immobilize, destroy or extract contaminants from soil and polluted water [18]. Phytoremediation has great potential to remove and degrade pollutants, transfer, destroy or stabilize elements considered harmful to the environment. It involves several groups of mechanisms related to a plant’s natural processes including phytoextraction (uptake of PTEs by plants and transportation to harvestable plant parts where they accumulate inside the plant organs), phytostabilisation or phytorestoration (pollutants including PTEs are removed from soil or water and transformed into a less toxic state), phytotransformation or phytodegradation (alteration of the chemical nature of pollutants into inactive, degraded or immobilized form using plant metabolism), phytostimulation (breaking down of pollutants by stimulating soil microbial activity in the soil often by symbiotic relationships of microbes with plant roots), phytovolatilisation (removal pollutants by converting them to less hazardous volatile compounds utilizing plants, in conjunction with transpiration process) and rhizofiltration (filtering water through a mass of roots to remove toxic substances or excess nutrients) [17, 19, 20].

Phytoextraction, is the most environmental friendly mechanism since it does not affect the soil structure/quality and is less expensive compared to the other clean-up processes [17]. It involves uptake of contaminants through the root system and storage in the root biomass and/or their transfer up into the stems and/or leaves. A living plant may continue to absorb contaminants until it is harvested. After harvesting, a lower level of the contaminant will remain in the soil, so the growth/harvest cycle is usually repeated through several crops to achieve a significant cleanup. After the process, the cleaned soil can support other vegetation or converted for agricultural purposes.

Papyrus vegetation growing in numerous wetlands has been associated with reclamation of contaminated soils in many countries [21]. Cyperus rotundus L. is one of the common papyrus vegetation occupying abandoned mines or areas where ASGM activities are practiced in western Kenya. It belongs to the sedge family Cyperaceae and is commonly referred to as papyrus sedge, paper reed, Indian matting plant, or Nile grass [21]. It is a perennial aquatic plant that is native to Africa, southern and central Europe, and southern Asia [17]. It forms reed-like swamp vegetation in many wetlands and grows to a height of about 55 inches. C. rotundus is an invasive weed which has been reported to spread out globally, both in tropical and temperate regions. This plant has a very long history of use by humans as a source of papyrus paper, animal feeds, for roofing, making mats and in boat building [17]. Medicinally, the plant is associated with numerous uses including the use of roots and tubers as antidote poisons, memory development, and harmonization of the liver, spleen, and pancreas [21]. The grass has been observed to exhibit anthelminthic, anti-fungal, insecticidal, anti-parasitic, anti-rheumatic, antispasmodic, aphrodisiac and astringent properties. It has also been used as a remedy for dyspepsia, vomiting, indigestion, thirst, worm troubles, cough, bronchitis and dysuria. In addition, it has been used in perfuming making [17].

Cyperus rotundus has been reported to be a good accumulator of Cd and Cr in an experiment where the plants were grown in pot plants containing simulated PTEs contaminated soil [17]. This plant has also been reported to be tolerant of high Cu levels since it has been observed to accumulate up to 300 mg/kg of Cu within its tissues without affecting the dry matter of the plant [22]. Studies in Philippines, Nigeria, Pakistan, Vietnam and Egypt have shown that C. rotundus accumulates more Pb metal in the shoots compared to the roots [23].

Excessive accumulation of PTEs in areas where mining is practiced results in contamination of agricultural soils which may lead to elevated PTEs uptake by crops, and thus affecting food quality and safety. It is therefore necessary to rehabilitate contaminated soil before conversion to agricultural use. Cyperus rotundus reed plants have been observed growing in swampy areas including abandoned artisanal small-scale gold mines in western Kenya. Some of the abandoned mines are converted to agricultural land for cultivation of crops and animal feeds. However, information on the potential Cyperus rotundus for cleanup PTEs including Cu, Pb and Cd which are harmful at elevated levels to both the environment and human health, from soil contaminated by ASGM activities is limited. This study is therefore aimed at determining the ability of C. rotundus in the cleanup of these metals from soil polluted by ASGM operations in western Kenya.

MATERIALS AND METHODS

Sample Collection

The study was carried out in Kakamega and Vihiga counties in western Kenya, where ASGM operations have been active over the years. The sampling localities were randomly selected to cover swampy areas with C. rotundus vegetation where ASGM activities have been halted. The sampling localities included Shibuye (0.2230° N, 34.8522° E), Sigalagala (0.1954° N, 34.7607° E) and Bushiangala (0.1861° N, 34.6833° E), in Kakamega County as well as in Muhudu 0.1622^0 N, 34.8374^0 E) and Kaimosi (0.1260° N, 34.8443° E) areas in Vihiga County. The control samples were collected from a site Virembe (0.2335^0 N and longitude of 34.87^0 E) next to Kakamega Forest where no mining activities have ever taken place. The plant samples were dug out using a soil auger in triplicate from each locality. Soil samples were dug out from the point of removal of the plant at a depth of 1-30 cm using the soil auger. Control samples for plant and soil were collected from Virembe the same way as the experimental samples. Replicate samples were collected at least 10 m apart in each locality. Both the plants and soil samples were stored in Ziplock plastic bags.

Sample preparation

The plant samples from the mine and control sites were washed and rinsed using deionized water to remove foreign matter such as soil and sand. The plants were separated into roots and leaves, which were cut into small pieces. Both the plant and soil samples were air dried in the sun for one week followed by oven drying (Labotec Ltd, Johannesburg, South Africa) for four hours at 100 ºC, after which they were separately ground into fine powder using hammer mill. A mass of 50 g of each replicate sample from the same locality were mixed thoroughly to form a composite sample.

Digestion of Soil and Plant Samples

A mass of 10 g sample of composite soil and plant part were ashed in a muffle furnace at 600 ^{0}C (Infitek Inc., Shandong, China) for 2 hrs. After ashing each sample was transferred into a digestion flask. A volume of 10 ml of conc nitric acid (65%, Analytical reagent: AR grade, Merck Ltd., Darmstadt, Germany) was added to each flask which was then covered and allowed to stand overnight. The contents of the beakers were heated on a hot plate (Labotec Ltd, Johannesburg, South Africa) at 125°C for one hour and then cooled to room temp. The digestion was continued at the same temp while adding 30% hydrogen peroxide (30%, AR grade, Merck Ltd., Darmstadt, Germany) until the digests were clear. The samples were heated to dryness at 80°C. Dilute nitric acid and deionized water were added in the ratio of 1:2 to dissolve the residues. Each sample was diluted to a final volume of 50 ml using deionized water. The samples were then filtered using a Whatman No. 40 filter paper and preserved for analysis of Cu, Cd, and Pb.

Determination of Copper, Lead and Cadmium Levels in Soil and Plant Samples

Determination of levels of the selected metals in soil and different plant parts of the plant samples from localities with abandoned mines and control samples was done using atomic absorption spectrophotometer (AAS). A series of standard solutions of Pb, Cd, and Cu used to generate a calibration curve for each metal were obtained from Scharlab Ltd. (Barcelona, Spain). The samples and standards were analyzed using an AAS.

Data Analysis

Statistical analysis including linear regression analysis, and calculation of means and standard deviation (SD) of replicate analyses, were performed with Microsoft Excel version 2013. A one-way analysis of variance (ANOVA, single factor without replication) and least significant difference (LSD) tests were applied to the data, and those results with $p \leq 0.05$ were considered to be significantly different. Pearson correlation was used to determine the effect of uptake of one element by the plant on the other. Bioconcentration factors (BCF) and translocation factors (TF) were determined using equations (i) and (ii) respectively [24, 25, 26].

$BCF = \frac{Corganism\ tissue}{Cabiotic\ medium}$………………Equation (i)

Where Corganism tissue is the concentration of a PTE in in the tissue of an organism (roots, stem and leaves) and Cabiotic medium is the concentration of the PTE in the abiotic medium (water, sediments or soil).

$TF = \frac{Caerial\ part}{Croots}$……………..……Equation (ii)

Where Caerial part is the concentration of the heavy metal in the shoot tissues (stem and/or leaves) and Croots is the concentration of a heavy metal in the roots of the plant.

The metal accumulation index (MAI) was used assess the overall metal accumulation capacity of plants and

was determined using equations (iii) and (iv).

$MAI = \sum_{j=1}^{N} Ij$………….Equation (iii)

$I_j = \bar{x}/\sigma$……………..Equation (iv)

Where N is the total number of metals analyzed and Ij is the sub-index for variable j, $\bar{x}$ is the mean concentration of an element and σ is its standard deviation [27].

Quality Control

To ensure reliability of results deionized water was used throughout the analysis and all reagents used were of AR grade. Calibration standards for each metal were used for instrument calibration and confirmation of the actual concentrations. Reagent blanks were used to apply corrections to the instrument readings. Validation of analytical results was achieved by conducting replicate analyses of the samples.

The recovery of each analyte was determined by means of spiking [28]. Pulverised samples (soils, roots, and leaves) collected from Kaimosi area were each subdivided into four portions, each weighing 1.00 g. One portion of each sample was spiked with a mixture of the three heavy metal standards (Cu, Pb, Cd), each at a conc of 5.0 µg/ml while the other two were spiked with the standard mixture at 10.0 and 20.0 µg/ml of each heavy metal respectively.

These spikes represented low, medium and high concentration of each analyte respectively. The fourth portion of each sample was not spiked with the standard mixture hence used as control samples. Both control and the spiked samples were digested as described in Section 2.3 and analysed using AAS. The recovery of each metal was expressed as the percentage concentration of the heavy metal recovered against the concentration of the relevant spike.

RESULTS AND DISCUSSION

Quality Control

The calibration curves for Cu, Pb and Cd were linear with R^2 values of 0.992, 0.998 and 0.995, respectively hence ideal for quantification of the PTEs in the soil and plant samples. Recovery data from AAS analyses of both spiked and unspiked soil, root and leaf samples from Kaimosi area is presented in Table 1. The recoveries of Cu, Pb and Cd were found to be in the ranges of 82.2–104%. These values were within the range of 80 to 120%, which is considered appropriate for analysis of PTEs in different samples [28].

Table 1: Results of recovery studies for copper, lead and cadmium in the soil and different parts of C. rotundus samples

Sample	Analyte	Sample content ($\mu g mL^{-1}$)	Spike ($\mu g mL^{-1}$)	Sample + Spike content ($\mu g mL^{-1}$)	Recovery content ($\mu g\ mL^{-1}$)	%Recovery
Soil	Cu	24.2	5.00	28.4	4.20	84.0
			10.0	33.9	9.70	97.0
			20.0	42.7	18.5	92.5
	Pb	24.4	5.00	29.3	4.89	97.7
			10.0	33.9	9.49	94.9
			20.0	43.1	18.6	93.4
	Cd	10.0	5.00	14.6	4.60	92.0

			10.0	19.5	9.50	95.0
			20.0	29.7	19.7	98.5
Roots	Cu	29.1	5.00	33.5	4.42	88.4
			10.0	38.1	9.02	90.2
			20.0	50.0	20.9	104
	Pb	41.4	5.00	46.4	4.96	99.1
			10.0	50.8	9.36	93.6
			20.0	59.8	18.4	91.8
	Cd	10.8	5.00	15.2	4.37	87.4
			10.0	19.6	8.77	87.7
			20.0	30.0	19.2	95.9
Leaves	Cu	25.6	5.00	30.1	4.54	90.8
			10.0	34.2	8.64	86.4
			20.0	44.5	18.9	94.7
	Pb	19.8	5.00	23.9	4.14	82.7
			10.0	29.2	9.44	94.4
			20.0	39.1	19.3	96.7
	Cd	9.29	5.00	13.4	4.11	82.2
			10.0	18.1	8.80	88.1
			20.0	28.9	19.6	98.1

Values represent mean±Sd of three replicates.

Levels of Potentially Toxic Elements in Soil and in Different Parts of Cyperus rotundus Samples

Phytoremediation has been used for rehabilitation of soil contaminated with PTEs using plants and ferns [29]. The plants absorb the PTEs from the soil, transport them and store them in plant parts that can be harvested easily. The data obtained from analysis of the Pb, Cu and Cd in soil and different parts of C. rotundus samples collected from different localities in western Kenya where ASGM activities have been stopped is presented on Table 2.

All the three PTEs were present in the soil and plant samples obtained from the different ASGM localities in elevated concentrations. The levels the PTEs in all the samples were in the order of Cu>Pb>Cd.

Table 2: Levels of PTEs in soil and different parts of C. rotundus samples collected from different localities.

Locality	**Conc (mg/kg of dry weight)**			
		Pb	Cu	Cd
Virembe-Control	Soil	Nd	0.208±0.030	Nd
	Roots	Nd	0.043±0.004	Nd
	Leaves	Nd	0.005±0.001	Nd
Bushiangala	Soil	76.5±4.61	132±3.53	71.2±5.30
	Roots	110±8.01	295±7.33	86.8±4.84
	Leaves	54.5±3.49	246±5.63	57.2±3.06
Sigalagala	Soil	173±9.72	206±4.45	24.8±2.68
	Roots	204±12.1	262±5.66	48.6±4.53
	Leaves	128±8.32	231±7.30	20.5±1.05
Shibuye	Soil	101±2.95	216±7.05	42.1±4.23
	Roots	141±9.22	242±6.43	69.7±4.10
	Leaves	65.0±3.24	241±5.18	34.6±4.79
Kaimosi	Soil	122±4.99	121±2.62	50.4±5.76
	Roots	207±5.27	134±2.66	54.2±7.20
	Leaves	98.8±4.41	128±3.14	46.4±2.72
Muhudu	Soil	208±8.81	203±3.75	37.7±1.42
	Roots	235±9.42	281±4.28	39.9±2.45
	Leaves	207±7.36	275±5.10	33.2±1.78
*FAO/WHO max permissible levels in Soil		85.0	36.0	0.800
*FAO/WHO max permissible levels in plants		2.00	10.0	0.020

Values represent mean±Sd of three replicates. Nd: Not detected. *FAO/WHO max permissible levels in soil and plants [31]

The concentration of the PTEs in the soil samples collected from the abandoned mines were significantly higher than those of the respective control samples ($p \leq 0.05$). The amounts of Pb and Cd in soil samples obtained from the control site were below the detection limits while the quantities of Cu were quite low

(0.208±0.030 mg/kg of dry weight). These findings are an affirmation that these metals are deposited on the earth's surface during excavation and processing of gold hence contributing to surface soil pollution by PTEs including Pb, Cu and Cd [30]. The amounts of each metal in the soil from abandoned mines were higher than the respective maximum permissible level for each metal in the soil samples according to WHO [31].

These results indicate that surface soil in areas where ASGM activities are practiced are not safe for agricultural use since they contain elevated levels of PTEs which pose danger both to plants, animal and human health. The levels of the Cu were highest in soil samples collected from the different localities followed by Pb and then Cd.

Quantities of the metals in the soil, root and leaf samples were in the order root>soil>leaf for the Pb and Cd while for Cu, the order was roots>leaves>soil in all the five localities. This indicates that Pb, Cu and Cd accumulate more in the roots compared to the leaves of C. rotundus. The levels of each of the three PTEs in the different parts of the plant samples obtained from the different locations were also above their respective maximum acceptable limits in plant samples [31]. Soil contaminated by the three metals had a significant impact on their respective levels in the roots, and leaves ($p \leq 0.05$).

Higher levels of each metal in the soil resulted in higher quantities in the roots and leaves of C. rotundus. Jahan-Nejati et al. [22] observed that higher level of Cu in soil samples resulted in higher levels in the C. rotundus plants. This data shows that C. rotundus has the ability of absorbing and Pb, Cu and Cd from soil and accumulating them in different parts hence has the potential for use in remediation of soil contaminated by these metals.

Although Cu is an essential element required for plant growth, Cu metal has been observed to be lethal to plants at concentrations >100 mg/kg [32]. The results obtained from this study are in agreement with those of Jahan-Nejat et al. [22]

who observed that C. rotundus is tolerant to high Cu concentrations where Cu stress of up to 300 mg/kg did not affect the plant. Copper is one of the poisonous PTEs that have been associated with nerve damage, stomach upset, sickness, and diarrhea at elevated levels [33].

Lead metal has been reported to cause damage to the brain and renal system, and also results in neurological and hematological defects [34]. The problems associated with Cd include lung damage, kidney diseases, reproductive defects, and defects in the growing fetus [35].

Bioconcentration and Translocation Factors of the Potentially Toxic Elements in C. rotundus and Soil Samples

The ability of a plant to be used for phytoremediation may be determined by its bio concentration factor (BCF) and translocation factor (TF) [24, 25, 26]. BCF is the ratio of the concentration of a heavy metal in the tissue of an organism to its relative concentration in the abiotic medium such as water, sediments or soil [34]. This ratio is used to evaluate the capacity of a plant to accumulate metals from substrates such as soil and water [29].

TF is a ratio that is used to show the ability of a plant to move PTEs from its roots to its aerial parts [25]. Plants which are hyper accumulators of PTEs have a strong tendency to take up the metals soil and translocate them into the above ground biomass, while tolerant plants usually limit soil-to-root and root-to-shoot transport of the metals leading to lower accumulation.

Phytoextraction is usually favored by plants with BCF and TF values greater than one [36]. Plants which exhibit BCF values greater than one and TF values less than one are potential phytostabilizers of the PTEs. The BCF and TF values obtained from the analysis of soil samples and different parts of C. rotundus obtained from different ASGM localities in western Kenya are presented in Table 3.

Table 3: Bioaccumulation factors and translocation factors for lead, Copper and Cadmium in roots, leaves of C. rotundus and corresponding soil samples

Element	**Locality**	**BCF**		**TF Leaves/Roots**
		BCF Root/Soil	BCF Leaves/Soil	
Pb	Virembe-Control	-	-	-
	Bushiangala	1.43±0.082	0.450±0.028	0.310±0.032
	Sigalagala	1.18±0.014	0.742±0.054	0.631±0.041
	Shibuye	1.20±0.061	0.640±0.021	0.463±0.007
	Kaimosi	1.70±0.068	0.806±0.056	0.484±0.018
	Muhudu	1.13±0.045	0.902±0.083	0.802±0.015
Cu	Virembe-Control	0.209±0.043	0.033±0.001	0.129±0.022
	Bushiangala	1.87±0.074	1.59±0.036	0.848±0.009
	Sigalagala	1.16±0.034	1.11±0.019	0.89±0.035
	Shibuye	1.14±0.088	1.10±0.048	0.992±0.027
	Kaimosi	1.21±0.032	1.06±0.036	0.881±0.038
	Muhudu	1.38±0.045	1.35±0.028	0.982±0.025
Cd	Virembe -Control	-	-	-
	Bushiangala	1.22±0.033	0.811±0.181	0.659±0.024
	Sigalagala	1.36±0.130	0.833±0.018	0.424±0.033
	Shibuye	1.25±0.012	0.822±0.220	0.504±0.067
	Kaimosi	1.07±0.044	0.923±0.014	0.858±0.031
	Muhudu	1.06±0.019	0.881±0.038	0.831±0.042

Values represent mean±Sd of three replicates.

The BCF values for all the PTEs were higher for the root samples compared to their respective leaf samples collected from the different ASGM localities. The BCF values for C. rotundus roots and leaves of the different metals were in the order Cu (1.12±0.09-1.87±0.07) > Pb (1.13±0.05-1.70±0.07) > Cd (1.06±0.02-1.36±0.10) and Cu (1.06±0.04-1.59±0.04) > Cd (0.81±0.18-0.92±0.01) > Pb (0.45±0.03-0.90±0.08) respectively. The TF values for Cu (0.85±0.01-0.99±0.03) were highest followed Cd (0.42±0.03-0.86±0.03) and lowest for Pb (0.31±0.03-0.80±0.01). This indicates that the roots accumulated higher levels of each metal compared to the leaves.

In addition, the roots of the plant take up the PTEs from the soils and further transport them to the leaves. The BCF values of the roots of each plant were greater than one indicating that the plants were good accumulators of the PTEs in the roots. Only the BCF value of Cu in the leaves was greater than one indicating that the amounts of Cu in the leaves were greater than those in the soil hence this plant can be regarded as a good accumulator of Cu in the leaves.

Since the BCF values of the roots of C. rotundus samples obtained from different ASGM localities for Cu, Pb and Cd were greater than the respective TF values, this plant can potentially be regarded as a good soil stabilizer of the three metals. Therefore the higher levels of each metal in the root tissues compared to the leaf tissues indicates that C. rotundus can stabilize the soil contaminated with Cu, Pb and Cd.

Ariyachandra et al. [20] observed that roots of C. rotundus to absorbed and accumulate the PTEs from the polluted soil at a higher rate than the shoots. Subhashini et al. [17] obtained BCF values of 44.18 and 4.42 for cadmium and chromium respectively, an indication that C. rotundus is an efficient accumulator for the two metals. C. rotundus has been observed to be a good accumulator of Cd since it accumulated up to 16.87 mg/kg within 60 days [17]. Sailakshmi et al. [23] observed that from studies conducted Nigeria, India, Malaysia, Vietnam, Pakistan, and Egypt indicated that that the roots of C. rotundus accumulate higher levels Pb compared to the shoots.

According to Ariyachandra et al. [20] Cyperus rotundus can be utilized as a phytostabilizer for Zn, Pb, and Ni. Phytostabilization reduces the movement of metals, leaching into groundwater and bioavailability of metals for entrance into the food chain. This plant has also been reported to be a Cd metal indicator due its ability to accumulate metals in the roots and transport them to their shoots, hence the quantities of this metal in the shoots is a reflection of the amount in the soil [20].

C. rotundus has been reported to be a Cu tolerant plant since it has been observed tolerate copper stress of up to 300 mg/kg of soil and accumulating Cu in the roots, tubers, and shoots without affecting the dry matter of the plant [22]. C. rotundus has the potential for use in phytoremediation of soils containing Pb, Cu and Cd, in areas with ASGM operations because it has been observed to exhibit high metal tolerance, high bioconcentration factor, has short cycle of life, high propagation rate, wide distribution and large shoot biomass [37].

Metal accumulation index of C. rotundus samples from different localities

The MAI values for C. rotundus samples collected from different localities are presented in Table 4. The MAI values were lowest in the control site (5.00) and ranged from 66.6 - 101 in the areas where ASGM activities were previously active. These results indicate that C. rotundus plants growing on soil contaminated by ASGM activities are enriched in PTEs relative to those in growing on unpolluted soil. High MAI values are important indicator for plants species selection in phytoextraction [38].

Therefore, C. rotundus has great potential for remediation of ASGM sites due to high MAI values. These results were in agreement with the findings of similar studies by Hu et al., [38].

Table 4: MAI values for C. rotundus samples obtained from different localities

Locality	Metal Accumulation Index (MAI)
Virembe-Control	5.00
Bushiangala	78.0
Sigalagala	66.6

Shibuye	73.8
Kaimosi	80.2
Muhudu	101

Correlation of levels of the Potentially Toxic Elements in the soil and different parts of C. rotundus samples

The quantities of PTEs in the roots and leaves of the plant were correlated with the respective levels in the soil samples as shown in Table 5. The correlation analysis data showed that the quantities of the PTEs in the roots and leaves of C. rotundus were correlated with those in the soil samples to varying degrees. The mobility and accumulation of metals in a growth medium is usually affected by competition between the different ions in the biota [39].

Table 5: Correlation matrix for levels of the PTEs in the soil and different parts of C. rotundus samples

	Pb_S	Pb_R	Pb_L	Cu_S	Cu_R	Cu_L	Cd_S	Cd_R	Cd_L
Pb_S	1.00								
Pb_R	0.953	1.00							
Pb_L	0.963	0.893	1.00						
Cu_S	0.827	0.799	0.692	1.00					
Cu_R	0.799	0.753	0.756	0.935	1.00				
Cu_L	0.808	0.752	0.786	0.957	0.990	1.00			
Cd_S	-0.263	-0.413	-0.119	-0.427	-0.605	-0.753	1.00		
Cd_R	-0.283	-0.402	-0.070	-0.706	-0.720	-0.692	0.922	1.00	
Cd_L	-0.303	-0.470	-0.171	-0.425	-0.785	-0.735	0.993	0.893	1.00

*n=54, p=0.05. Pb_S (conc of Pb in soil), Pb_R (conc of Pb in roots), Pb_L (conc of Pb in leaves), Cu_S (conc of Cu in soil), Cu_R (conc of Cu in roots), Cu_L (conc of Cu in leaves), Cd_S (conc of Cd in soil), Cd_R (conc of Cd in roots), Cd_L (conc of Cd in leaves). Concentrations are in mg/g of dry weight.

The correlation analysis showed strong positive correlations between the amounts of metals in soil for Pb_S and Cu_S (0.827) and weak negative correlations for Pb_S and Cd_S (-0.263) and Cu_S and Cd_S (-0.427). The relative amounts of each metal in the different plants depends on the amounts of that metal present in soil samples collected at the same point with the plant. Strong positive correlations between the PTEs in the soil and the respective root samples for Pb_S and Pb_R (0.953), Pb_S and Pb_L (0.963), Cu_S and Cu_R (0.935), Cu_S and Cu_L (0.957), Cd_S and Cd_R (0.922), Cd_S and Cd_R (0.993). This trend was also observed the correlation between the metal levels in the roots and leaves of the plants, Pb_R and Pb_L (0.893), Cu_R and Cu_L (0.990) and Cd_R and Cd_L (0.893). These findings indicate that the amounts of the PTEs that accumulate in the roots and leaves of C. rotundus plants growing in abandoned ASGM mines depend on the quantities present in the soil where the plant grows. The positive and significant correlations of Cu, Pb and Cd in soil and plant tissues of C. rotundus indicated their potential usage as a biomonitor for these elements ($p < 0.05$). These results are in agreement with Hosseini et al. [40].

PTEs in the soil and in different plant tissues interact with each other either synergistically or antagonistically. Synergistic relationships cause an increase in the uptake of one metal in the presence of another while antagonistic interactions result in the decreased uptake of one metal due to the uptake of another [41]. Synergistic interactions were observed between concentration of metals in the soils and different plant parts for Pb_S and Cu_R (0.799), and for Pb_S and Cu_L (0.808). These relationships were also observed for the metal levels in the roots and leaves for Pb_R and Cu_R (0.753), Pb_R and Cu_L (0.752), Pb_L and Cu_R (0.756), and, Pb_L and Cu_L (0.786). Antagonistic interactions were observed between Cu_S and Cd_R (-0.706), Cu_R and Cd_S (-0.605), Cu_R and Cd_R (-0.720), Cu_R and Cd_L (-0.785), Cu_L and Cd_S (-0.753), Cu_L and Cd_R (-0.692), and, Cu_L and Cd_L (-0.735). The uptake of Cu by plants results in increased uptake of Pb but decreased uptake of Cd from the soil by C. rotundus, while the uptake of Pb is not affected by availability of Cd in the soil and root tissues.

CONCLUSIONS

Potentially toxic elements including Cu, Pb and Cd pose a significant threat to environment as a result of their persistence in soils. These metals may enter the food chain hence causing serious health issues. ASGM activities result in deposition of Cu, Pb and Cd on the surface soil leading to soil contamination. C. rotundus plants absorb Cu, Pb and Cd from soil through the roots and translocate them to the leaves of the plant. The levels of the metals in the soil and accumulated in the different parts surpassed the acceptable limits by WHO. The amount of each metal accumulated in the roots was higher than those in the leaves. Cyperus rotundus plants growing on soil polluted by gold mining activities had higher quantities of the PTEs compared to those growing on unpolluted soil. There were strong positive correlations between the levels of the PTEs in the soil and the respective plant tissues indicating that the plants are good accumulators of the metals. The uptake of Cu by C. rotundus plants caused an increase in the uptake of Pb but resulted in decreased uptake of Cd from the soil by the plant. The uptake of Pb by C. rotundus is not affected by availability of Cd in the soil and root tissues. C. rotundus has great potential for use in rehabilitation of soil contaminated from mining activities by its ability to absorb Cu, Pb and Cd from the soil and accumulate them in its tissues. However, C. rotundus used for rehabilitation of contaminated soil should be destroyed and livestock should not be allowed to feed on these plants since minerals absorbed are harmful to both animals and humans. Phytoremediation of PTEs including Cu, Pb and Cd from soil contaminated by ASGM activities using C. rotundus is an environmentally friendly and cost-effective method that can be used for rehabilitation of soil by reduction of heavy metal toxicity.

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