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### Evaluation of Toxic Metal Accumulation in *Calotropis procera*

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#### ABSTRACT

*Calotropis procera* samples around municipal waste contaminated sites within Bauchi L.G.A were analyzed for heavy metals using the Atomic Absorption Spectrophotometer, in order to determine the ability of the plant species to bio-accumulate these metals. Results showed that mean concentrations of cadmium, chromium, copper, lead, except manganese and zinc in the aerial parts and root of the plant samples were higher than the normal ranges in plants and control site values. The weighted means of the studied metals in the plant in Yelwa were in the order: Zn (94.93mg/kg) > Mn (81.23mg/kg) > Pb (73.51mg/kg) > Cu (49.10mg/kg) > Cr (25.21mg/kg) > Cd (3.97mg/kg), while in Wunti Dada, the order was: Cu (68.67mg/kg) > Mn (53.67mg/kg) > Pb (48.53mg/kg) > Zn (43.07mg/kg) > Cr (19.94mg/kg) > Cd (9.95mg/kg). ANOVA results indicated that there were no significant differences among the average concentration factors (CFs) of Cu, Pb, Mn and Zn in the plant studied in both contaminated sites. Cu has the highest CF values of  $0.71 \pm 0.07$  mg/kg while that of Cd ( $0.21 \pm 0.03$ ) was the lowest. The order for the translocation ratio (TR) for the metals in the plants were Pb (2.81) > Mn (2.22) > Cr (1.92) > Zn (1.14) > Cu (0.88) > Cd (0.23). The high TRs values for these metals are an indication that the plant has the potential to phyto-extract these metals from contaminated soils.

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#### INTRODUCTION

The term "heavy metal" is commonly used to categorize metals and metalloids associated with pollution and toxicity. They are a class of trace metals with density greater than  $5.0 \text{ g cm}^{-3}$  (Okeke *et al.* 2014a). These metals are very important contaminants in the environment, originating from anthropogenic activities such as industrial processes, agricultural practices, and naturally from the weathering of rocks (Adeniyi *et al.* 2008; Bi *et al.*, 2020; Jiang *et al.*, 2020; Kumuduni *et al.*, 2022).

The accumulation of these heavy metals in the soil, from which they are transferred into living organisms via ground water and the food chain, has remained a matter of growing environmental concern (Parven *et al.*, 2009, Sarwar *et al.*, 2017; Liu *et al.*, 2020; Zhang *et al.*, 2021). Once these metals are released into the environment, they are not readily removed by metabolic activities; they therefore accumulate and cause serious environmental pollution (Lasat, 2002; Nwuche and Ugoji, 2008; Muhiuddin *et al.*, 2010).

The physical and chemical methods proven to mitigate these metals in the environment have been applied for the remediation of soil at a small scale. On a large scale, these methods are expensive and could affect the soil quality for sustainability. Suitable technique such as Phytoaccumulation could be employed to remove contaminants from soils. Phytoaccumulation, which is a subset of phytoremediation is referred to as botanical bioremediation, and involves the use of green plants to decontaminate soils by the accumulation of metals taken up by plant root system (Chaney *et al.*, 1997). The metal ions accumulated in the aerial parts of the plants can be removed to dispose off or burnt to recover the metals. Phytoremediation can be considered because of its long-term applicability and cost effectiveness, in addition to other benefits (Kannan and Kanimozhi, 2009) and can be explored in reclaiming polluted soils.

Several plants have been reported for their phytoremediation abilities. These include Alfalfa, *Brassica Junicea* (Indian mustard green), ordinary tomatoes, maize plant, poplar, Sudan grass, sunflowers, scented geranium and duckweed (Uwumarongie *et al.*, 2008; Parven, 2009; Oyelola *et al.*, 2009). Baskeran *et al.*, 2009 reported that *Azadirachta indica* plant grown on sugar mill effluent polluted soil for 120 days was able to accumulate 0.63ppm Cu, 4.52ppm Zn, 6.37ppm Fe and 2.5ppm Mn. The potentials of *Azadirachta indica* and *tamarindus indica* to bioaccumulate heavy metals have also been reported by other researchers (Okeke *et al.*, 2014a, 2014b).

*Calotropis procera*, a species of plant in the dogbane family, Apocynaceae is native to North Africa, West Asia and Indonesia (GRIN, 2021). It is a small evergreen shrub (10-15 feet high). The green globes are hollow but the flesh contains a toxic milky sap that is extremely bitter and turns into gluey coating resistant to soap (Kaur *et al.* 2021). The plant is claimed to have

pharmacological applications (Sailendra *et al.*, 2002; Adamu, 2005; tour and Talele, 2011; Al-Rowaily *et al.* 2020, Mehmood *et al.*, 2020; Nadeem *et al.*, 2019) and can be used as a bioinsecticide, biofungicides and possible herbicides (Kaur *et al.* 2021).

The plant has been reported to possess phytoremediation potentials. *Calotropis procera* plants growing around Mahad AD'Dahab mine has been observed to accumulate Arsenic (Al-Farraj and Al-Wabel, 2007). While research carried out by Abida *et al.*, (2009) revealed that the root of *Calotropis procera* plant is the best of biomass selected for the phytoaccumulation of Arsenic from waste water; a similar study carried out using biomass prepared from roots of this plant (*C. procera*) has shown that the absorbent could be used to remove Cu (II) and other heavy metals from industrial waste water (Hifsat *et al.*, 2010). Gajbhiye *et al.* (2019) noted that the plant can be used for environmental biomonitors for the assessment of metals. However, little or nothing has been documented on the phytoaccumulation of heavy metals by this plant species with respect to waste contaminated sites in Bauchi, Nigeria. In this study, the concentration factors of some selected heavy metals and their transfer ratios were investigated with a view to determine the metal accumulation potential of *Calotropis procera* around waste contaminated soils in Bauchi municipal.

## MATERIALS AND METHODS

The study was carried out in municipal waste contaminated sites in Yelwa (YD) and Wuntin Dada (WD) areas of Bauchi. Geographically, Bauchi state lies between latitudes 9° 30' N and 12° 34' N and longitude 8° 5' E and 11° 00' E of the Greenwich meridian. It occupies a total area of 549,259.01 sq. kilometers representing about 5.3% of the land mass of Nigeria. The state spans two vegetation zones namely Sudan and Sahel Savannah. Effective rains start

in mid may or sometimes around early June and ends in late October. The dry season starts in October and ends in May. The average annual rainfall is between 1000mm and 1300mm.

### Sampling and analysis

Three randomly selected *Calotropis procera* plants within each of the study sites were used for this study. The leaves, shoots and roots of the plants were collected and stored in paper bags. These aerial parts were then washed thoroughly with running tap water to remove the soil particles. The samples were cut into smaller pieces and oven- dried at 60°C for three (3) days; ground to powder and stored in labeled polythene bags (Oyelola et al., 2009; Pahalawattaarachchi et al., 2009).

Three replicate samples were digested with a mixture of conc. nitric acid and perchloric acid. 1.0g of the ground dried samples was placed in a small beaker and 10ml of the conc.  $\text{HNO}_3$  was added to the beaker and allowed to stand overnight. The contents were heated on a hot plate in a hood until the production of red  $\text{NO}_2$  fumes has ceased. The beaker was cooled and 3.5ml of 70%  $\text{HClO}_4$  was then added. The mixture was heated and allowed to evaporate to a small volume. The digested samples were diluted to 50ml with distilled water (Perkin- Elmer, 1996). Each sample extract were then placed in plastic bottles, before subjecting them to analysis of the six metals using the Atomic Absorption Spectrophotometer (A Analyst 400, Perkin Elmer, U.S.A).

Top soil samples were collected from ten (10) different locations on each of the study areas at soil depth of 0 – 30cm using a soil auger and stored in polyethene bags. The samples were thoroughly mixed

together, conned and quartered several times before the required samples for analysis were obtained. The samples were then air dried, crushed in a mortar and sieved through a 2mm sieve (Uwumarongie et al., 2008). Three (3) replicate samples of 5.0g of the air-dried, ground and sieved sample were accurately weighed and placed in labeled stopper container. 20ml each of 0.05N HCl and 0.025N  $\text{H}_2\text{SO}_4$  were added and placed in a mechanical shaker for 15mins. The mixture were filtered into a 50ml volumetric flask and diluted to the mark with the mixture of the acids (Perkin Elmer, 1996). The digested samples were then subjected to analysis of the six metals using the Atomic Absorption Spectrophotometer (Analyst 400, Perkin Elmer, U.S.A).

### RESULTS AND DISCUSSION

The heavy metal contents of *Calotropis procera* plant tissues and the weighted mean concentration in the various study sites are shown in Table 1. Table 2 shows the transfer ratios of the heavy metals from the root to the leaves and the shoots, and the concentration factors of the metals under investigation.

#### Accumulation of metals in *Calotropis procera*

Table 1 shows the accumulation patterns of the studied heavy metals in the leaves, shoot and root of *Calotropis procera*. Cd and Cu accumulation in this plant was as follows, leave< shoot< root. Pb and Mn accumulated in the order leave< root< shoot. The accumulation of Cr and Zn in the plant parts in both sites showed almost similar pattern, only that the level of the Cr in the shoots and roots are not significantly different in WD. Pb and Mn accumulated significantly in the bark compared to the leaves and root.

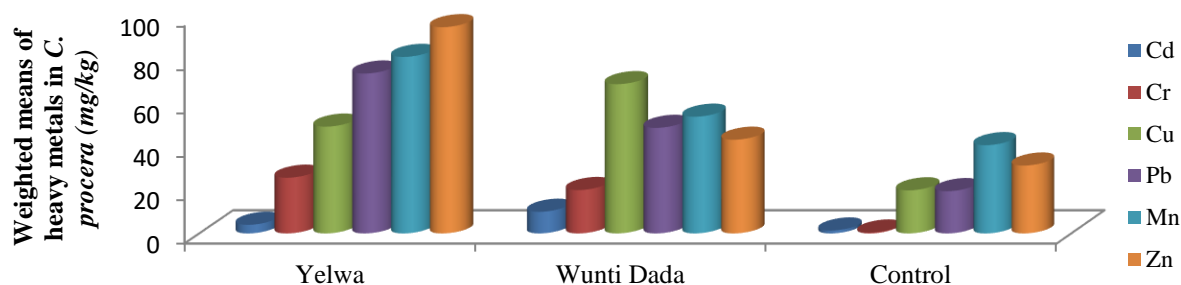
Table 1: Accumulation of Heavy Metals (mg/kg) in *Calotropis procera*

METAL	YELWA			WUNTIN DADA			CONTROL SITE			NORMAL RANGE IN PLANTS
	Leave	Shoot	Root	Leave	Shoot	Root	Leave	Shoot	Root	
Cd	0.67 <sup>a</sup> ±0.29	3.02 <sup>b</sup> ±0.28	8.22 <sup>c</sup> ±0.23	ND	0.16 <sup>a</sup> ±0.01	19.72 <sup>b</sup> ±3.17	0.36 ±0.82	1.47 ±0.47	2.19 ±0.19	* 0.1-2.4
Cr	12.38 <sup>a</sup> ±2.81	39.25 <sup>b</sup> ±1.47	25.20 <sup>c</sup> ±0.87	10.20 <sup>a</sup> ±1.51	28.23 <sup>b</sup> ±0.50	21.40 <sup>b</sup> ±6.00	0.15 ±0.82	0.75 ±0.23	0.30 ±0.13	*** 0.03-14
Cu	26.05 <sup>a</sup> ±2.60	50.65 <sup>b</sup> ±5.30	70.60 <sup>c</sup> ±4.50	31.45 <sup>a</sup> ±2.30	52.50 <sup>b</sup> ±7.31	122.00 <sup>c</sup> ±11.20	16.50 ±1.60	20.65 ±2.10	22.25 ±4.56	* * 5- 20
Pb	33.12 <sup>a</sup> ±2.70	139.40 <sup>b</sup> ±3.78	48.13 <sup>c</sup> ±2.05	32.45 <sup>a</sup> ±2.46	65.33 <sup>b</sup> ±0.48	47.82 <sup>c</sup> ±1.01	6.64 ±0.66	29.33 ±1.04	22.32 ±2.52	*** 0.2 -20
Mn	32.78 <sup>a</sup> ±3.73	135.30 <sup>b</sup> ±13.40	75.57 <sup>c</sup> ±5.16	31.98 <sup>a</sup> ±1.30	78.93 <sup>b</sup> ±2.59	50.05 <sup>c</sup> ±0.50	12.78 ±3.73	65.48 ±1.04	43.40 ±7.20	* 20-1000
Zn	48.80 <sup>a</sup> ±3.36	101.80 <sup>b</sup> ±1.57	134.20 <sup>c</sup> ±6.59	15.47 <sup>a</sup> ±0.90	54.05 <sup>b</sup> ±0.66	59.70 <sup>b</sup> ±6.71	14.68 ±1.06	35.20 ±0.87	44.00 ±1.18	* * 1-400

(Okeke et al., 2014b, ND – Not detected, Values within a row with different superscripts are significantly different (p< 0.05).

The weighted mean metal accumulations in this plant on the study sites in Figure 1 were generally above the control samples and normal range of the metals in plants shown in Table 1, except for Mn and Zn. This shows the plant has the ability to accumulate Cd, Cr, Cu and Pb. The weighted

means of the metals in the whole plant in YD was in the order: Cd<Cr<Cu<Pb<Mn<Zn, while a different order with respect to Cu and Zn was observed in WD and control site: Cd<Cr<Zn<Pb<Mn<Cu.

Figure 1: Accumulation pattern of the metals in *C. procera* on contaminated Sites

This pattern of accumulation of the study metals may be associated with the large genetic differences that exist among similar plant species; coupled with the fact that the rate and extent of movement of metal ions within plants do not only depend on the metal concern, but also on the plant organs and age of plant (Alloway, 1996). The

results obtained in this study are consistent with researches that report the ability of *C. procera* to accumulate several metals including Pb, Cr, Cd, Mn, Cu (Almendi *et al.*, 2019; Kaur *et al.*, 2021). The level of Cd in this study is within the values reported in Mohammed et al (2011).

### Ability of *C. procera* to absorb metal in the contaminated sites

Table 2 presents the average translocation ratio (TR), which is the ratio of the concentration of heavy metal in the aerial parts of a plant to that in the root; and explains the mobility of metals from the root to the aerial parts of a plant (Pahalawattaarachchi *et al.*, 2009). The TR from root to leaves for Pb was observed to be highest in this plant species. Although absorption from soil is a possible way to increase Pb concentration in

leaves, yet Markert *et al.* (1993), suggested that aerial deposition and foliar uptake may contribute significantly to Pb accumulation in leaves. The order of TR in the leaves was  $Cd < Zn = Cu < Cr < Mn < Pb$ . The total TR value (0.23) of Cd in the leaves in this work is lower than values reported for *Talinum triangulare* (1.23 and 1.26) in Eze (2014); *Azadirachta indica* (56.17) in Okeke (2014a) and *Tamarindus indica* (13.67) in Okeke, (2014b).

Table 2 Average Translocation Ratio (TR) of Metals to Leaves and Barks of *Calotropis procera* in the study sites

Metal		Cd	Cr	Cu	Pb	Mn	Zn
Average Translocation Ratio (TR)	(root to leaves)	0.04	0.48	0.31	0.68	0.54	0.31
		±0.06	±0.01	±0.08	±0.01	±0.15	±0.07
	(root to Shoot)	0.19	1.44	0.57	2.13	1.68	0.83
		±0.25	±0.07	±0.20	±1.00	±0.15	±0.11
Total TR (Aerial parts)		0.23	1.92	0.88	2.81	2.22	1.14
		±0.31	±0.08	±0.28	±1.01	±0.30	0.18

Values within the row (for the CF values) with different superscripts are significantly different ( $p < 0.05$ ).

Several studies have indicated that Cd is readily translocated to other parts of the plants, especially the leaves after absorption by the roots, yet there are other evidences that Cd is present in higher concentrations in the roots than other parts of tomatoes, soya beans, maize, oats and alfalfa (Alloway, 1996). The reason for this could be attributed to the fact that other elements in the soil affect Cd absorption. The relative excesses of metals such as Mn, Cu, Ni and Se have the ability to reduce the uptake of Cd in plants. Zn has been considered to have an antagonistic effect on the uptake of Cd in soils with low concentration of Cd (Alloway, 1996), as is the case in this study. These explanations may be the reasons for the low TR

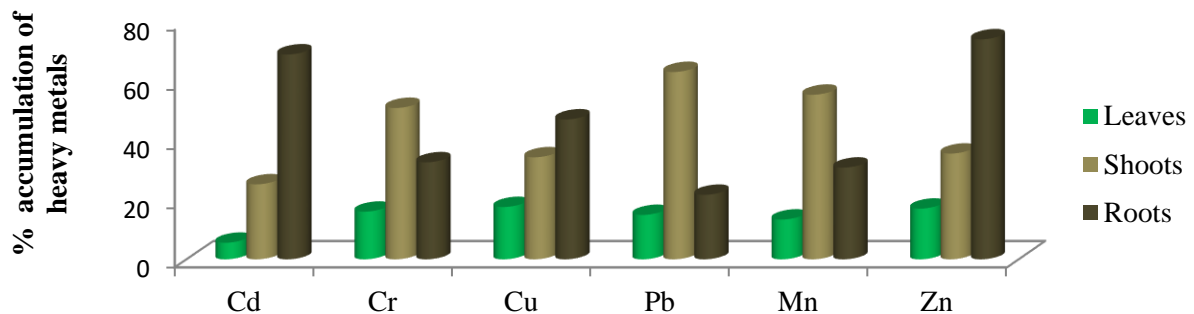
recorded for Cd in this work. In the shoots, metal translocation ratios also increased in the order  $Cd < Cu < Zn < Cr < Mn < Pb$ . These results are probably related to the differences in the solubility and availability of each ion. All the studied metals except Cd had total TRs  $> 1$ , indicating that the plant has the potential for the phyto-extraction of these metals from contaminated soils. The low total TR for Cu (0.88) in relation to other metals is comparable to those reported in Eze (2014) (Cu: 0.66 - 0.68). Kabata-Pendias and Henryk (1984) explained that the root tissues of plants have the potential to hinder the transport of Cu and Zn to shoots under conditions of deficiency or excess of both metals.

Table 3 Average Concentration Factor of *C.procera*

Metal	Cd	Cr	Cu	Pb	Mn	Zn
Average Concentration Factor (CF)	0.21 <sup>a</sup> ±0.03	0.39 <sup>b</sup> ±0.38	0.71 <sup>c</sup> ±0.07	0.37 <sup>b</sup> ±0.09	0.38 <sup>b</sup> ±0.12	0.37 <sup>b</sup> ±0.16

The concentration factor (CF), which is the ratio of metal in the plant to the concentration of metal in the soil ( $C.F = [HM]_{\text{Plant}} (\text{mg/kg}) / [HM]_{\text{Soil}} (\text{mg/kg})$ ) is shown in Table 3. This factor gives an idea of the ability of a plant to accumulate the metals absorbed from the soil and reveals the accumulation pattern in the plants. In addition, CF quantifies the relative differences in the bioavailability of metals to plants (Pahalawattaarachchi, *et al.*, 2009). The CFs for the six elements detected in *C.procera* in both contaminated sites in Table 2 shows that Cu has the highest value ( $0.71 \pm 0.07$ ), while Cd had the lowest ( $0.21 \pm 0.03$ ). The CFs for the other metals are not significantly different. The average CF value

of Cd obtained for *C.procera* in this study is lower than those reported in Al-Farraj and Al-Wabel (2007) (1.84) but were higher for Cu and Pb, when compared to those reported by Al-Farraj and Al-Wabel (2007) (0.28 for Cu and 0.11 for Pb); and the ranges reported in Mirela (2000) (0.01-0.1 for Cu and Pb). The lower CF values recorded in this study for most of the metals could be due to the fact that the CFs were calculated on the basis of total metal concentration in the soil rather than on the bio-available fraction. Using the bio-available fractions of the metals in the soil is most likely to give higher CF values for this plant. *C.procera* therefore exhibits the capacity to absorb and accumulate all the study metals in the soil.

Figure 2: Percentage accumulation of metals by each plant component of *C. procera* in Yelwa

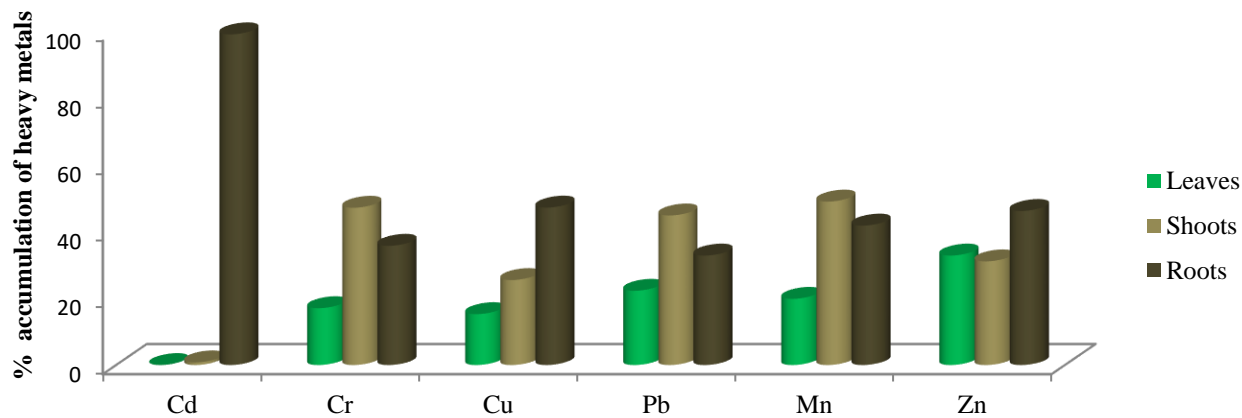


Figure 3: Percentage accumulation of metals by each Plant component of *C. procera* in Wuntin Dada

A very large percentage of the all the metals studied in this plant species accumulated mostly in the roots and shoots (Figures 2 and 3). This is an indication that the leaves are prevented from contamination and may not cause any serious environmental pollution. Considerable amount of these metals would therefore be prevented from re-entering the biogeochemical cycle or transport to the adjacent water bodies, when the leaves eventually fall from the tree (Pahalawaachchi *et al.*, 2009).

Table 4: Pearson Bivariate Correlation Coefficient for Metals in *C. procera* (whole plant)

	Cd	Cr	Cu	Pb	Mn	Zn
Cd	1					
Cr	.835(*)	1				
Cu	.694	.929(**)	1			
Pb	.662	.943(**)	.982(**)	1		
Mn	.395	.569	.590	.549	1	
Zn	.009	-.104	.099	-.049	.573	1

\* Correlation is significant at the 0.05 level \*\* Correlation is significant at the 0.01 level

**Pearson Bivariate Correlation Coefficient for Metals in *C. procera* (whole plant)**

A significant positive relationship was observed between the levels of Cd and Cr ( $r=0.835$ ;  $p<0.05$ ), while a highly significant positive relation exists between Cr and Cu ( $r=0.929$ ;  $p<0.01$ ) and for Cr and Pb,  $r=0.943$  at  $p<0.01$  as shown (Table 3). The nature of correlation in this study with regards to Cd aligns with Alloway, (1996). In his study, while the relative excesses of metals like Mn and Cu could reduce the uptake of Cd in plants, Zn is considered to have an antagonistic effect on the uptake of Cd in soils with low concentration of Cd, as is the case in this study.

### Concentrations of Heavy Metals in the Study Sites.

The heavy metal concentrations in the contaminated sites were well over those of the control samples (Fig. 4). Oyelola *et al.* (2009) observed that the municipal wastes dumpsite in Isolo, Lagos, accumulated more of Pb, Cr, Cu, Fe and Zn, while levels of Cu and Cr in soils obtained from Bendel wood treatment factory operating for more than 40 years were very high, when compared to soils collected away from the wood treatment factory (Uwumarongie *et al.*, 2008). The concentrations of Cd, Cr, and Pb are almost

similar to those reported by Oguntimehin and Ipinmoroti (2008) in automobile waste contaminated soils in Akure. The reduced levels of some of the metals in this study, when compared to the control may be as a result of the volume and nature of wastes generated in the sites, physiochemical properties of the soil *e.t.c.* (Iwegbue *et al.*, 2006). The order of abundance of the metals in YD and Control site (C.S) were Zn>Mn>Pb>Cu>Cr>Cd while in the WD, the order was Mn>Zn>Pb>Cu>Cd>Cr.

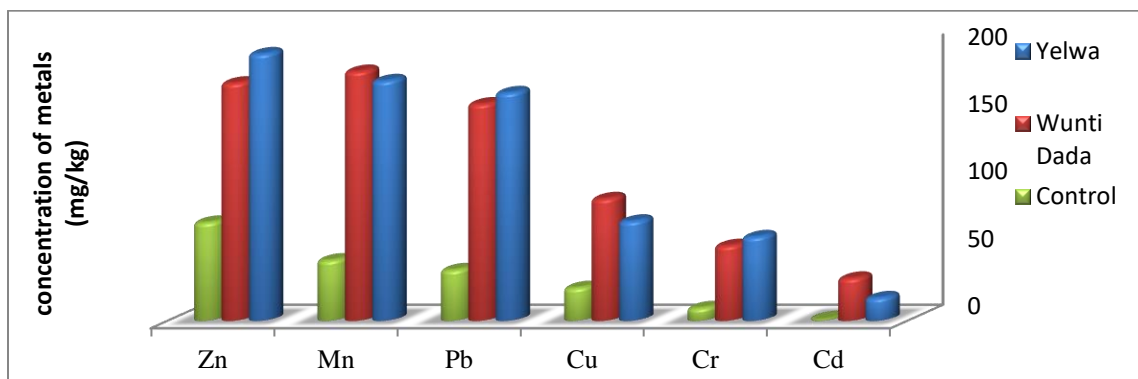


Figure 4 Accumulation of heavy metal in the contaminated sites and control site

### CONCLUSION AND RECOMMENDATION

The study revealed that the concentration factors for all the metals studied in *Calotropis procera* ranged from moderate to high, except for Cu ( $0.71 \pm 0.07 \text{ mg/kg}$ ). The high CF value for Cu is an indication that the plant can be used for the bioaccumulation of this metal. The low concentration (CF) of Cd may be attributed to the fact that Cd is toxic to plants, hence plays no important roles in plant metabolism. Interesting, all the metals studied in this plant accumulated mostly in the roots and barks, preventing the leaves from contamination. Based on the findings

made, the following recommendations are therefore considered necessary.

1. *Calotropis procera* plants should be grown in and around metal contaminated sites to serve as trap for these metals.
2. More research should be carried out on other plants to determine their Phytoaccumulation potentials.

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