Namibian Journal for Research, Science and Technology

Vol 1, October 2018

Namibia Journal for Research, Science and Technology

# Prevalence and Chemo-kinesis of Toxic Trace Metals in Environmental Samples

I. Hilia[1], C. Hange[2], F. Hakala[3], M. Matheus[4], C. Jansen[5], J. Hidinwa[6] and O. Awofolu[7]*

[1-7]Department of Health Sciences, Namibia University of Science and Technology, Windhoek, Namibia
Corresponding author: (O.Awofolu) oawofolu@nust.na; 061-207-2500

ARTICLE INFO

*Article History:*

Received: July 2017
Published: October 2018

*Keywords:*

Trace metals, pollution, human health, prevalence, samples, ICP-OES

ABSTRACT

The aim of the study was to assess incidences, level and mobility of some toxic trace metals in environmental samples. By this, the potential impacts of anthropogenic activities on environmental and human health would be evaluated. Soil, plant and lower animal samples were randomly collected from stratified study area, labelled and taken to the laboratory for pre-treatment and analysis. Acid digestion technique was employed for the isolation of metallic contents in samples and quantitation was by Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-OES). The analytical protocol was validated through the quality assurance process which was found acceptable with quantitative metallic recoveries in the range of 85-90 %; hence considered applicable for the analyses of samples. The mean concentration of analysed metals in soil samples ranged from 53.2- 2532.8 mg/kg (Cu); 59.5- 2020.1 mg/kg (Zn); 1.80 – 21.26 mg/kg (Cd) and 19.6-140.9 mg/kg (Pb). The mean level in grass samples ranged from 9.33 – 38.63 mg/kg (Cu); 64.20-105.18 mg/kg (Zn); 0.28–0.73 mg/kg (Cd) and 0.53 -16.26 mg/kg (Pb) while the mean level in lower animal sample (beetle) varied from 9.6 - 105.3 mg/kg (Cu); 134.1-297.2 mg/kg (Zn); 0.63 – 3.78 (Cd) and 8.0 – 29.1 mg/kg (Pb) across sample collection points (SCPs) 1-4 respectively. Metallic transfer factors (TFs) were in the order Zn >Cd > Cu> Pb with metal Pollution Indices (MPIs) in the order SCP1 > SCP2 > SCP3 > SCP4. About 60-70 % of analysed metals were above the Maximum Allowable Limits (MALs) in soil and plant samples using the European Economic Commission (EEC) and CODEX MALs respectively. Results obtained revealed general prevalence of analysed metals at all sampled sites with indication of metallic mobility across the food chain. This signifies dire consequences for environmental and human health. Control of pollution from source and passive environmental remediation strategies are recommended.

## 1. Introduction

The prevalence of toxic heavy metals such as Cadmium (Cd), Arsenic (As), Lead (Pb), Zinc (Zn), Copper (Cu) and others in the ecosystems at elevated levels continues to be of great concern in view of the health implications across environmental strata. Although the earth crust contains natural level of these metals (Singh et al. 2011), anthropogenic activities have introduced substantial amount into the environment at an unprecedented rate (Armah et al. 2014). Some of these metals such as Cd and As have no physiological benefits, hence their possible transfer across the food chain to human forms the basis of concern. This is due to the fact that Cd is a known endocrine disruptor in human (Kartenkamp 2011) while lead (Pb) has been implicated in the disruption of gene expression (Gillis et al. 2012), hence the incessant interest in monitoring the trend and distribution of these metals in the various ecosystems mainly for health purposes.

Apprehension about the prevalence of these toxic metals in the environment is exacerbated by their potential for intra- and inter-ecosystem mobility. Soil for example, has been described as a repository of heavy metals (Cai et al. 2012). In spite of this, the bioavailability and distribution of these metals within the ecosystem is influenced by certain factors and conditions. Hence, the form in which these metals exist determines their availability.

Some physico-chemical variables such as pH have been reported to influence the bioavailability of these metals in soil (Kashem and Singh 2001). Once bio-available, they can migrate among various organisms within the terrestrial (soil) ecosystem and possible further migration into the aquatic ecosystem. Erosional process of surface soil for example has been reported to be responsible for the transfer to heavy metals into the aquatic ecosystem (Dan et al. 2014).

In this study however, the interest lies in studying possible kinesis or mobility of trace metals from the soil ecosystem into indicating organisms whose interaction and sustenance depend on soil either from habitational or food source point of view. This is significant in that it will reveal the heavy metal burden of the soil possibly from anthropogenic input and the vulnerability of living organisms that depend on it. It will further show the distribution trend of the toxic metals in indicator organisms and the potential for inter-ecosystem migration with consequential health effects across the food chain. The process involves systematic and periodical collection of indictor organisms in order to establish migration and trend as well as the soil itself and then analysed for their metallic content.

Therefore, this study aimed at investigating possible prevalence of selected toxic trace metals (Cd, As, Pb, Zn and Cu) in soil and their transfer to indicating organisms that depend directly or indirectly on the soil. This was with a view of examining possible transfer across the food chain and the health implications.

## 2. Materials and Methods

### 2.1 Study area

The study was conducted within the local municipal township of Tsumeb, located in the Northern part of Namibia close to the Etosha National Park in the Oshikoto Region.The town is at an altitude of 1, 266 m, latitude -19, 2333 (1913'59.880''S) and longitude 17, 7167 (1743'0.120''E). It has a population of about 19 840 and a total area of 271 km$^2$ (NSA, 2011). Major commercial activities in the area include agriculture, mining, metal foundry and construction. Therefore, the study was carried out in this area as a result of potential impacts of these anthropogenic activities on the environment and human health. The study area was stratified into four stratum and samples collected randomly from each stratum named as sample collection points (SCPs). Hence the location of SCP 1:S19$^o$ 13' 58.8"; E017$^o$ 42' 35.7"; SCP 2: S 19$^o$ 14' 41.7"; E 017$^o$43' 12.0"; SCP 3: S19$^o$ 15' 21.6"; E 017$^o$ 42' 08.5" and SCP 4: S19$^o$ 15' 38.5"; E 017$^o$ 42' 43.2".

### 2.2 Samples and sampling process

In this study, metal indicator samples used were the insect (Stag beetle) and plant together with the soil samples. The plant sample was the African Foxtail grass (*Cenchrus ciliaris*) as identified by a qualified botanist. This plant grow wildly and is readily available in the study area, hence considered as good indicator while the invertebrate was the Stag beetle e.g. *Rhinotia hemistictus.* Samples were collected from the four (4) different sites represented as SCP1, SCP2, SCP3 and SCP4 as stated earlier between the period of July and October 2015 using stratified random sampling for the soil and plant samples within each stratum. Purposive sampling from each stratum was however conducted for the invertebrate which was specifically searched for as a result of availability and collected for analysis.

Soil samples to the depth of about 100 mm were collected randomly from each site with the aid of clean stainless steel soil trowel. The trowel was washed and rinsed properly with water after each sampling. Soil and plant samples were placed in transparent plastic zipper bags, labelled and taken to the laboratory for further treatment and analysis. The invertebrate samples were collected by digging into the soil debris, particularly under large trees and stones within each site, placed in the zipper bags, labelled and taken to the laboratory for further treatment and analysis.

### 2.3 Sample treatment and analysis

Deposits of soil particles on plant and invertebrate samples were removed by rinsing gently with tap water and then with distilled water. The plants were cut into smaller pieces, placed in crucible and dried in oven at 120$^o$C for 24 h. The invertebrate samples were also placed in crucibles and also dried in a similar manner. The dried samples were grinded in clean mortar and pestle and then sieved using 0.63 µm sieve. Soil samples were oven dried at 30$^o$C and also sieved. All metal determinations were based on the final fine powdery samples. Analysis of metallic content in environmental samples followed a previously described method by Awofolu (2005). Quality assurance of the analytical process was by standard metal addition and quantification in all cases was by Inductively-Coupled Plasma Emission Spectroscopy (ICP-OES).

**Table 1: Heavy metals transfer factors (TFs) across sample collection points**

| SCPs | Trace Metals | | | |
|---|---|---|---|---|
| | Cu | Zn | Cd | Pb |
| SCP 1 | 0.02 | 0.05 | 0.58 | 0.12 |
| SCP 2 | 0.11 | 0.75 | 0.11 | 0.05 |
| SCP 3 | 0.31 | 1.16 | 0.16 | 0.10 |
| SCP 4 | 0.18 | 0.96 | 0.15 | 0.03 |

The bold values are those closer to or > 1

## 2.4 Statistical analysis

Correlation between analysed heavy metals from each sample collection point (SCP) was determined in order to verify possible relationship amongst them using the MS excel. The heavy metal transfer factor (TF), expressed as the ratio of the metal level in soil to that of the plant within the SCP was also determined in order to reveal the accumulation pattern of metals by the plant. The metal pollution indices (MPIs) were determined as the ratio of heavy metal concentration obtained at study site to that from the control site. This is important in order to establish the extent of pollution/ contamination of the site. MPI values > 1 express the extent of pollution of the site while values < 1 indicates the contamination range. The two ranges contain further sub-divisions that indicate the scale of impact as shown in Table 2.

**Table 2: Metal pollution/contamination indices (MPIs) and pollution significance across sampling sites**

| **SCPs** | **Heavy metals** | | | |
|---|---|---|---|---|
| | **Cu** | **Zn** | **Cd** | **Pb** |
| **SCP 1** | **15.6 (8.1 – 16.0)** Very severe pollution (VSP) | **22.1 (>16)** Excessive pollution (EP) | 3.80 (2.1 - 4.0) Moderate pollution (MP) | **117.4 (>16)** Excessive pollution (EP) |
| **SCP 2** | 0.83 (0.76-1.0) Very severe contam. (VSC) | 1.10 (1.1-2.0) Slight pollution (SP) | 0.74 (0.5-0.75) Severe contam (SC) | **51.7 (>16)** Excessive pollution (EP) |
| **SCP 3** | 0.24 (0.1-0.25) Slight Contam (SC) | 0.65 (0.5-0.75) Severe contam. (SC) | 0.32 (0.26-0.5) Moderate contam (MC) | **18.1 (>16)** Excessive pollution (EP) |
| **SCP 4** | 0.33 (0.26-0.5) Moderate contam (MC) | 0.73 (0.5-0.75) Severe contam (SC) | 0.33 (0.26-0.5) Moderate contam (MC) | **16.3 (>16)** Excessive pollution (EP) |

**Significance adapted from (Haliru et al., 2014); Bold values are those of concern**

## 3.0 Results

The quality assurance of an experimental process is important in order to check the applicability of the method for sample analysis. Hence, the result of this process presented by percentage metal recoveries from standard metal additions were in the range of Cd (78 ± 0.002); Pb (89 ± 0.005); Cu (90 ± 0.003) and Zn (87 ± 0.01).

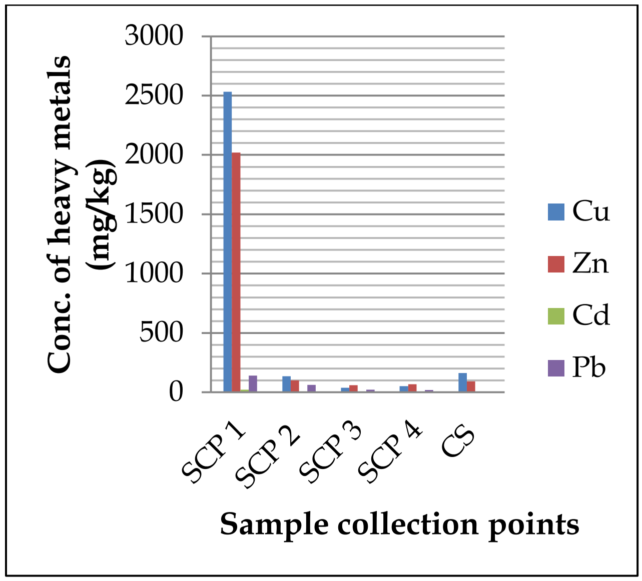


**Fig 1:** Mean conc. (mg/kg) of heavy metals in soil samples across the sampling points; CS = Control Site

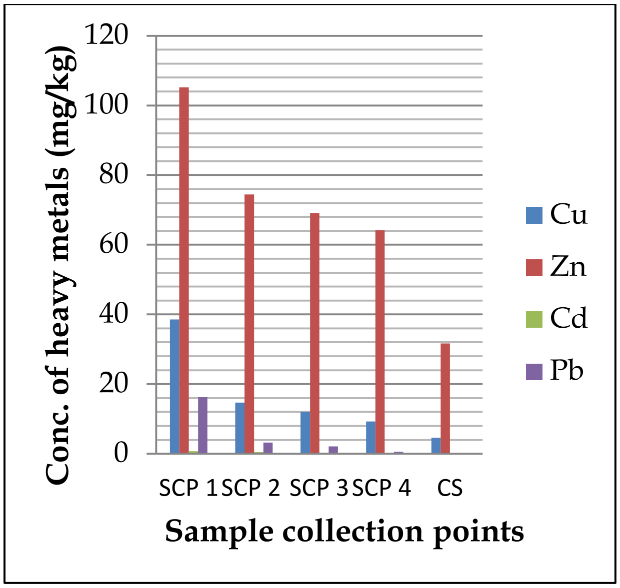


**Fig 2:** Mean conc. (mg/kg) of heavy metals in grass samples across the sampling points; CS = Control Site

The quality assured method was then applied to the determination of heavy metals in samples. The mean concentration of heavy metals in soil samples (Figure 1) for Cu at SCP1, SCP2, SCP3 and SCP4 were 2533 ± 25.3 mg/kg; 134.8 ± 11.4 mg/kg; 38.9 ± 4.81 mg/kg, 53.2 ± 5.50 mg/kg respectively while the value for control site (CS) was 162.2 ± 9.83 mg/kg. The value for Zn varied from 2020.1 ± 41.6 mg/kg; 99.7 ± 12.6 mg/kg; 59.5 ± 3.7 mg/kg and 66.9 ± 2.97 mg/kg with CS value of 91.4 ± 10.3 mg/kg. The mean concentration for Cd was 21.3 ± 2.13 mg/kg; 4.2 ± 0.41 mg/kg; 1.8 ± 0.12 mg/kg and 1.78 ±0.53 mg/kg with CS value of 5.6 ± 0.87 mg/kg. Lastly, the mean concentration of Pb varied from 140 ± 12.4 mg/kg; 62.0 ± 3.2 mg/kg; 21.7 ± 1.17 mg/kg and 19.6 ± 2.21 mg/kg also with CS level of 1.20 ± 0.13 mg/kg; all across sampling period and SCP sequence as above.

The mean concentrations of heavy metals in *Cenchrus ciliaris*) are presented in Figure 2. The mean values of Cu ranged from 38.6 ± 3.40 mg/kg; 14.7 ± 2.13 mg/kg; 12.0 ± 1.12 mg/kg and 9.3 ± 2.01 mg/kg with the CS value of 4.6 ± 0.18 mg/kg. The mean concentration of Zn ranged from 105.2 ± 9.41 mg.kg; 74.4± 4.32 mg/kg; 69.1 ±2.84 mg/kg and 64.2 ±2.16 mg/kg with CS mean value of 31.7 ± 2.37 mg/kg. The mean level of Cd were 0.73 ± 0.31 mg/kg; 0.45 ±0.14 mg/kg; 0.28 ± 0.11 mg/kg and 3.0 ± 0.08 mg/kg with CS value of 0.05 ± 0.02 mg/kg while the mean level for Pb varied from 16.3 ± 1.52 mg/kg; 3.2 ± 0.23 mg/kg; 2.12 ± 0.13 mg/kg and 0.53± 0.15 mg/kg also with CS value of 0.05 ± 0.03 mg/kg; all respectively at SCP1, SCP2, SCP3 and SCP4 across the sampling periods.

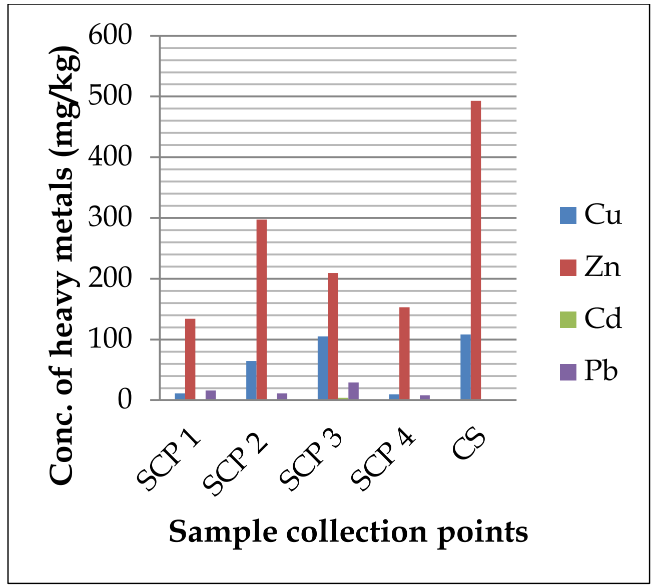


**Fig 3:** Mean conc. (mg/kg) of heavy metals in insect (Stag beetle) across the sampling points; CS = Control Site

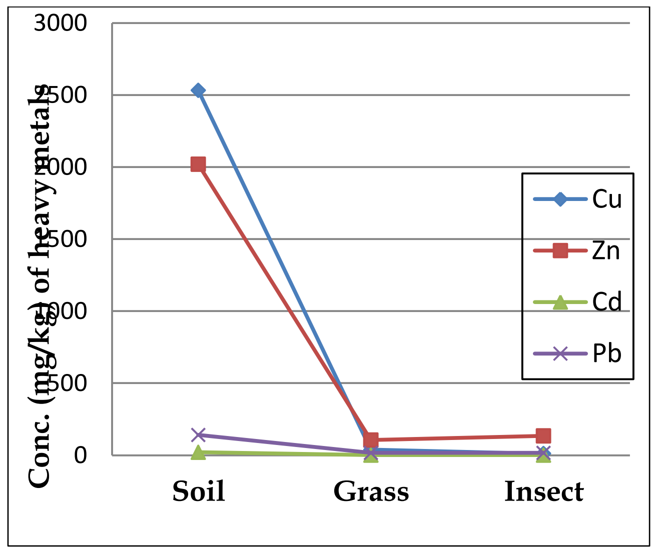


**Fig 4:** Mean conc. trend of heavy metals (mg/kg) in all samples at sample collection point 1 (SCP1)

For the analysed metals in invertebrate (Stag beetle) samples, the mean concentration of Cu varied from 11.3 ± 0.20 mg/kg; 64.5 ± 2.11 mg/kg; 105.3 ± 9.51 mg/kg; 9.6 ± 0.10 mg/kg with CS value of 108.1 ± 12.1 mg/kg. The level of Zn ranged from 134.1 ± 11.4 mg/kg; 297.2 ± 21.2 mg/kg; 209.4 ± 17.3 mg/kg and 152.8 ± 13.6 mg/kg while the value at the CS was 492.9 ± 23.9 mg/kg. The mean level for Cd ranged from 0.91 ± 0.18 mg/kg; 1.41 ±0.07 mg/kg; 3.78 ± 0.13 mg/kg and 0.63 ± 0.12 mg/kg with CS of 0.45 ± 0.08 mg/kg. Lastly, the level of Pb varied from 15.9 ± 2.17 mg/kg; 11.4 ± 0.54 mg/kg; 29.1 ± 0.11 mg/kg and 8.0 ±0.35 mg/kg with the

CS value of 0.53 ±0.05 mg/kg; all respectively at SCP1, SCP2, SCP3 and SCP4 across the sampling periods.

**Table 3: Analysis of correlation between analysed metals in samples at each SCP**

| *SCPs* | | *Cu* | *Zn* | *Cd* | *Pb* |
|---|---|---|---|---|---|
| 1 | **Cu** | 1 | | | |
| | **Zn** | 0.999745 | 1 | | |
| | **Cd** | 0.999855 | 0.999985 | 1 | |
| | **Pb** | 0.999978 | 0.999873 | 0.999946 | 1 |
| | | *Cu* | *Zn* | *Cd* | *Pb* |
| 2 | **Cu** | 1 | | | |
| | **Zn** | 0.005654 | 1 | | |
| | **Cd** | 0.984482 | -0.16991 | 1 | |
| | **Pb** | 0.956434 | -0.28654 | 0.992824 | 1 |
| | | *Cu* | *Zn* | *Cd* | *Pb* |
| 3 | **Cu** | 1 | | | |
| | **Zn** | 0.942386 | 1 | | |
| | **Cd** | 0.986583 | 0.875127 | 1 | |
| | **Pb** | 0.880112 | 0.670582 | 0.945814 | 1 |
| | | *Cu* | *Zn* | *Cd* | *Pb* |
| 4 | **Cu** | 1 | | | |
| | **Zn** | -0.47139 | 1 | | |
| | **Cd** | 0.979071 | -0.28204 | 1 | |
| | **Pb** | 0.923661 | -0.09744 | 0.982321 | 1 |

**SCP = Sample Collection Point**

The calculated transfer factor (TF) for the heavy metals (Table 1) were in the range of 0.02-0.31 (Cu); 0.05-1.16 (Zn); 0.11-0.58 (Cd) and 0.03-0.12 (Pb) across SCP1 to SCP4 respectively. The Metal Pollution Indices (MPIs) of analysed heavy metals as shown in Table 3 ranged from Cu (0.24-15.6); Zn (0.65-22.1); Cd (0.32-3.80) and Pb (16.3-117.4). The various ranges obtained will shed more light into the extent of contamination of analysed metals at the sample collection sites. It is also imperative to investigate possible relationship between the analysed metals at the various sample collection points (SCPs). At SCP1, near perfect correlation (r = 0.999) were obtained between the analysed metals while at SCP2 the correlation between the metals varied from -0.16991 to 0.992. At SCP3 the metal correlation spectrum were from 0.67- 0.99 while at SCP4, the correlation varied from -0.47 to 0.98.

## 4.0 Discussion

The focus of the study was to assess the prevalence of heavy metals of toxicological potential in soil and to examine possible mobility or transfer of these metals to some living organisms whose sustenance hinged on this ecosystem. Although trace metals are said to be natural constituents of the earth's crust, elevated levels in the environment have been attributed to anthropogenic activities (Tchounwou et al. 2012). Highest mean value of 2533 mg/kg; 2020.1 mg/kg; 21.3 mg/kg and 140 mg/kg, all at SCP1 were obtained for Cu, Zn, Cd and Pb respectively in soil samples which were significantly higher (in the order of magnitude of 16, 20, 4 and 117 respectively) than the mean values obtained at control sites (CS) for these metals. They were also higher than the maximum permissible limits (MPLs) of 50–140 mg/kg of Cu; 150–300 mg/kg of Zn; 1-3 mg/kg of Cd with the exception of 50-300 mg/kg of Pb in soil (EEC 1986). This outcome confirmed the notion of soil as a sink for heavy metals. The high level of analysed metals in soil obtained in this study was an indication of substantial soil enrichment of these metals. This would mostly likely have occurred as a result of anthropogenic input. Possible sources of these metals are from atmospheric deposition from mining operations, metals foundry and deposition of petrochemical wastes on land. The high metallic level obtained in this study was not unusual. Similar high concentrations of 469 mg/kg of Pb and 404.4 mg/kg of Cu in soil within the vicinity of a cement factory have been reported (Ogunkunle and Fatoba 2014).

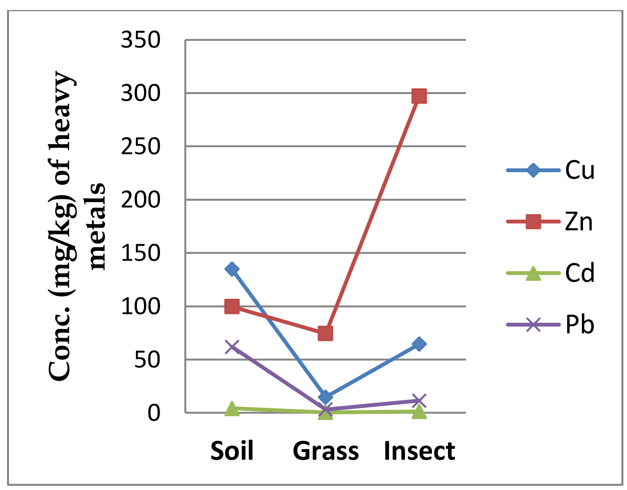


**Fig 5:** Mean conc. (mg/kg) trend of heavy metals in samples at sample collection point 2 (SCP2)

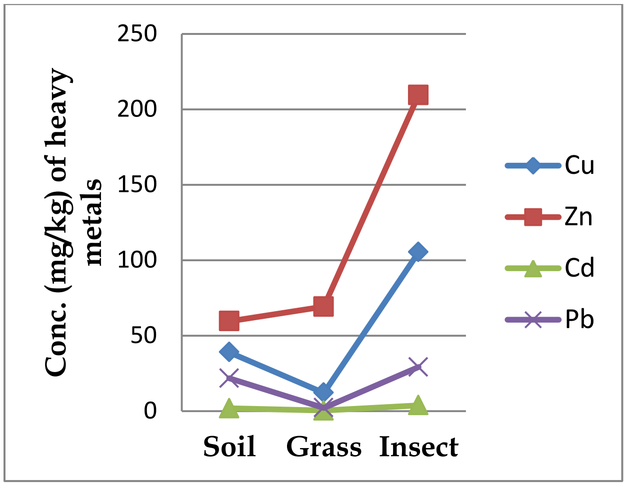


**Fig 6:** Mean conc. (mg/kg) trend of heavy metals in samples at sample collection point 3 (SCP3)

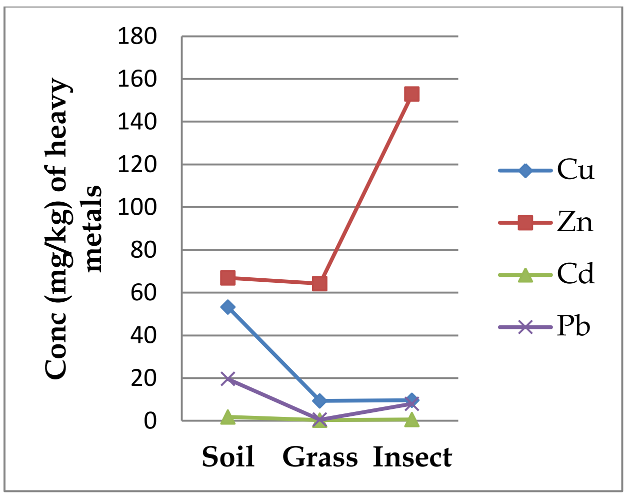


**Fig 7:** Mean conc. (mg/kg) trend of heavy metals in samples at sample collection point 4 (SCP4)

It is generally known that some living organisms depend on the soil for either sustenance, habitation or both. Hence, this study revealed the mobility of the metals from the soil to plants and lower animal as a result of their interaction with the soil ecosystem as shown in Figures 4 -7. Possible accumulation of some of these metals can be observed with higher level of Zn in the lower animal at SCP2 (Figure 5); Zn and Cu at SCP3 (Figure 6) and Zn at SCP4 (Figure 7). The level of heavy metals obtained in plant samples was generally lower than those in soil and the insect. The plant's accumulating capacity was relatively lower when compared to similar study where higher accumulation tendency was reported (Chen 2010). The low accumulation may also be due to the effect or influence of soil conditions such as conductivity and pH (Mleczek et al. 2009). The highest mean level of Cu obtained in plant samples was lower (0.38 mg/kg) than the maximum allowable limit (MAL) of 73 mg/kg of Cu in plants while those recorded by Zn, 100 mg/kg; Cd, 0.1 mg/kg and Pb, 0.3 mg/kg were higher than the recommended values (CODEX 2001). Although, there were no standard of limits for metals in invertebrates, the detection of higher levels of Zn (Figure 5, SCP2), Cu and Zn (Figure 6, SCP3) and Zn (Figure 7, SCP4) relative to other samples are an indication of migration and high pollution of the ecosystem. Accumulation of heavy metals in insect species relative to the soil has been reported (Zia et al. 2015). Higher levels of Pb (1,243 mg/kg) and Cd (575.6 mg/kg) in insect have also been reported in a previous study (Zhong-Sheng et al. 2009).

Generally, the mobility of trace metals from the soil into the plants and insects in the study has been established. The vertical mobility of heavy metals from soil to plants in the vicinity of mining activities was also reported (Nkongolo et al. 2013).

Metal transfer factor, as expressed by the soil-to-plant transfer is one of the key components of human exposure to heavy metals through the food chain. The accumulation of heavy metals in plants predisposes their transfer to ruminants and eventually to human. Transfer factor value close to or greater than 1 is an indicative of high transfer/accumulation of metals by the plant. In this study, moderate TF values of 0.75 (SCP2); high TF of 1.16 (SCP3) and 0.96 (SCP 4) were obtained for Zn while an average TF value of 0.58 at SCP1 was obtained for Cd as shown in Table 1. High TF values of 1.11 (Cd) and 0.89 (Pb) have also been reported in a similar study (Aktaruzzaman et al. 2013). Generally, these high TFs are quite worrisome in view of the health implications across the food chain.

Elevated levels of toxic heavy metals in the environment have been linked to anthropogenic activities. Hence, Metal pollution indices (MPIs) are usually determined in order to establish this veracity. The MPI standard ranges and their significances, adapted from (Haliru et al. 2014) together with values obtained in this study are as presented in Table 2. Of great concern were the very severe pollution index of 15.6 within the range of (8.1 – 16.0) for Cu; excessive pollution index of 22.1 for Zn (> 16) at SCP1 and also excessive pollution for Pb (> 16) for SCP1 to SCP4. These are indicative of anthropogenic input of the heavy metals into the environment.

Correlation analyses are widely applied to establish possible relationship between variables as well as similarity of their pollution sources in environmental studies (White et al. 2005; Li et al. 2012). High correlation between heavy metals in analysed substrates could be a reflection of similar pollution sources of the metals (Manta et al. 2002; Zou 2015). In this study, the heavy metals correlation analysis revealed near perfect correlation (r = 0.999) between all analysed metals at SCP1; very strong correlation between Cu and Cd (r = 0.98) and Cu and Pb (r = 0.95) as well as Cd and Pb (r = 0.99) at SCP2. At SCP3, strong correlation was also observed between Cu and Zn (r = 0.94); Cu and Cd (r = 0.99) as well as Cd and Pb with (r = 0.95). The correlation between Cu and Cd (r = 0.98); Cu and Pb (r = 0.92) and Cd and Pb (r = 0.98) were considered very strong. All these are indicative of strong association and interaction between these metals and hence similar sources in the environment as shown in Table 4. However, Zn and Cd (r = - 0.17) and Zn and Pb (r = - 0.29) at SCP2 were found to be negatively correlated.

## 5.0 Conclusion

The prevalence of analysed toxic heavy metals in soil was revealed in the study as well as possible migration or transfer to environmental indicators such as plant and lower invertebrate organism (insect) within the soil ecosystem. The outcome generally confirmed the occurrence of the analysed metals above permissible levels. The mobility or kinesis of heavy metals from soil to plant and the insect was established through ~ and > 1.0 TF values obtained for some of the analysed metals. This is quite worrisome in that possible bio-magnification across the food chain is highly possible resulting in exposure and threat to human health. Consequently, control of pollution from source and use of passive remediation strategies are recommended.

**Acknowledgement**

Financial resources provided by the National Commission for Research, Science and Technology (NCRST) and the Namibia University of Science and Technology (NUST) are appreciated.

## References

Aktaruzzaman M, Fakhruddin ANM, Chowdhury MAZ, Fardous Z, Alam MK. Accumulation of Heavy Metals in Soil and their Transfer to Leafy Vegetables in the Region of Dhaka Aricha Highway, Savar, Bangladesh. Pakistan Journal of Biological Sciences, 16, 332-338 (2013).

Armah FA, Quansah R, Luginaah I. A Systematic Review of Heavy Metals of Anthropogenic Origin in Environmental Media and Biota in the Context of Gold Mining in Ghana. International Scholarly Research Notices, 2014, 1-37 (2014).

Awofolu OR. A survey of trace metals in vegetation, soil and lower animal along some selected major roads in metropolitan city of Lagos, 105, (1-3), 431-47 (2005).

Cai L, Xu Z, Ren M. Source identification of eight hazardous heavy metals in agricultural soils of Huizhou, Guangdong Province, China," Ecotoxicology and Environmental Safety, 78, 2–8 (2012).

Chen YE, Yuan S, Su YQ, Wang L. Comparison of heavy metal accumulation capacity of some indigenous mosses in Southwest China cities: a case study in Chengdu City. Plant, Soil Environ. 56, 60–66 (2010).

CODEX STAN 223. General standard for vegetables Codex Alimentarius Commission Joint FAO/WHO Food Standards Programme Food and Agriculture Organization of the United Nations, Rome Italy, Vol 13 (2001).

Dan SF, Umoh UU, Osabor VN. Seasonal variation of enrichment and contamination of heavy metals in the surface water of Qua Iboe River Estuary and adjoining creeks, South-South Nigeria. J Oceanography and Marine Sci. 5, 6, 45-54 (2014).

EEC. European Economic Commission. European Commission Office for Official Publications of the European Communities, Luxembourg. Council Directive 86/278/ EEC on the protection of environment and in particular of soil, when sewage sludge is used in agriculture (1986).

Gillis BS, Gavin IM, Arbieva Z. Analysis of lead toxicity in human cells. BMC Genomics, 13, 344 (2012).

Haliru HA, Ling LP, Selaman OS. Environmental Burden of Heavy Metal Contamination Levels in Soil from Sewage Irrigation Area of Geriyo Catchment, Nigeria. Civil and Environmental Research, 6, 10, 118-124 (2014).

Kartenkamp A. Are cadmium and other heavy metal compounds acting as endocrine disrupters? Met Ions Life Sci. 8, 305-17 (2011).

Kashem MA, Singh BR. Metal availability in contaminated soils: I. Effects of floodingand organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn**.** Nutrient Cycling in Agroecosystems, 61, 3, 247-255 (2001).

Li X, Liu L, Wang Y, Luo G, Chen X, Yang X, He X. Integrated Assessment of Heavy Metal Contamination in Sediments from a Coastal Industrial Basin, NE China. *PLoS ONE*, *7*, 6 (2012), e39690 doi.org/10.1371

Manta DS, Angelone M, Bellanca A, Neri R, Sprovieri M. Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. The Science of the Total Environment, 300, 229–243 (2002).

Mleczek M, Magdziak Z, Rissmann I, Golinski P. Effect of different soil conditions on selected heavy metal accumulation by *Salix viminalis* tissues. J Environ. Sci & Health, Part A, 44, 14, 1609-1616 (2009).

Nkongolo KK, Mehes-Smith M, Narendrula R, Cholewa E. Mobility of heavy metals in plants and soil: A case study from a mining region in Canada. Am J Environ Sci. 9, 6, 483-493 (2013).

NSA. Namibia Statistics Agency, Government of the Republic of Namibia. The Namibia Population and Housing Census Report. DDI_NAM_NSA_PHC_2011_v01 _PUMS, Windhoek, Namibia (2011).

Ogunkunle CO, Fatoba PO. Contamination and spatial distribution of heavy metals in topsoil surrounding a mega cement factory. Atmospheric Pollut. Res. 5, 2, 270-282 (2014).

Singh R, Gautam N, Mishra A, Gupta R. Heavy metals and living systems: An overview. India J Pharmacol. 43, 3, 246-253 (2011).

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metals Toxicity and the Environment**.** EXS, 101, 133-164 (2012).

White HK, Xu L, Lima ANL, Egliton TI, Reddy CM. Abundance, composition and vertical transport of PAHs in marsh sediments. Environ. Sci. and Technol. 39, 8273–8280 (2005).

Zhong-Sheng Z, Xian-Guo L, Qi-Chao W, Dong-Mei Z. Mercury, Cadmium and Lead Biogeochemistry in the Soil–Plant–Insect System in Huludao City. Bull Environ Contam Toxicol. 83, 255–259 (2009).

Zou J, Dai W, Gong S, Ma Z. Analysis of Spatial Variations and Sources of Heavy Metals in Farmland Soils of Beijing Suburbs. *PLoS ONE*, *10, 2 (2015)* e0118082. http://doi.org/10.1371