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Determination of Heavy Metal and Macronutrients in *Hyperiodrilus africanus* (Earthworm) and *Scolopendra cingulata* (Centipede) at Coal Mining Sites in Enugu State, Nigeria

***[1]Ogbonna, P.C., [2]Nzegbule, E.C. and [3]Okorie, P.E.**
[1,2]Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[3]Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
*ogbonna_princewill@yahoo.com

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ABSTRACT

Earthworms and centipedes from two mined sites were analyzed for heavy metals (Ni, Pb, Cd, Fe, As) and macro-nutrients (Ca, Mg, K, N, P). Highest concentrations of Ni (18.94 ± 0.74 mg/kg), Pb (13.01 ± 0.07 mg/kg), and Fe (580.00 ± 1.41 mg/kg) was observed in earthworm while Cd (0.22 ± 0.04 mg/kg) and As (0.005 ± 0.001 mg/kg) were highest in centipede during wet season at Iva mine. Levels of Ni, Pb, Fe, Cd, and As in Akwuke mine ranged from 5.48 ± 6.11 (dry season) to 10.10 ± 0.71 (wet season), 0.84 ± 0.06 (dry season) to 8.04 ± 0.07 (wet season), 122.80 ± 3.96 (dry season) to 396.00 ± 1.56 (wet season), 0.03 ± 0.01 (wet season) to 0.12 ± 0.08 (wet season) and 0.001 ± 0.000 (wet season) to 0.003 ± 0.001 (wet season) mg/kg respectively which was lower than 4.20 ± 0.13 to 18.94 ± 0.74, 1.73 ± 0.06 to 13.01 ± 0.07, 151.20 ± 3.11 to 580.00 ± 1.41, 0.01 ± 0.00 to 0.22 ± 0.04 and 0.002 ± 0.014 to 0.005 ± 0.001 mg/kg respectively at Iva mine during the wet season. Highest content of Ca (6.01 ± 0.04 cmol/kg), Mg (190.60 ± 2.83 cmol/kg), K (150.00 ± 2.26 cmol/kg), and N (1.28 ± 0.18 cmol/kg) were recorded in earthworm while P (3.04 ± 0.08 cmol/kg) was higher in centipede in wet season at Akwuke mine. The use of metal-contaminated earthworm is a route of entry of heavy metals along the food chain.

1. INTRODUCTION

Soil is an important component of the terrestrial ecosystem in which living organisms such as plants and animals (including soil fauna) thrive. The soil provides plants with the essential nutrients for growth and development while soil fauna is provided with organic material for food (e.g. earthworm) as well as cover or protection from predators. Plants, in turn, replenish the soil nutrients via shedding of their leaves and other plant parts (Ogbonna *et al*., 2010) while soil fauna (e.g. earthworm) improves soil structure through burrowing (Bhadauria and Saxena, 2010) that alter the physical structure of the soil and also improves soil aeration for organisms living (Jones *et al*., 1994) in the soil as well as enhances plant root penetration (Culliney, 2013) and growth (Lal, 1999; Scheu, 2003). Earthworms, like all creatures, are part of food webs, thus providing food for predators. Centipedes break down dead plants and animals and turn them into soil nutrients. Processes occurring in the soil are very crucial in sustaining productivity of the terrestrial ecosystem (Ogbonna *et al*., 2010).

Coal is a hard black mineral found in coalface where it is cut out of the rock (Ogbonna *et al*., 2018a). Coal mining as a land use has the capacity of changing ecological processes because of the nature of the mineral, method of exploitation and the extensive area involved and the accumulation of contaminants in soils (via mine tailings) that may reach levels of concern (Ogbonna *et al*., 2015). The increasing rate of soil pollution over the last decades is constituting a serious risk to man and the ecosystem via direct inhalation of metals contaminated dust and dermal contact with contaminated soil, or indirect contact through the food chain (soil-plant-human or soil-plant-animal-human) (Ogbonna *et al*., 2015).

Substantial air pollution takes place at mining sites during excavation and transportation (Ogbonna *et al*., 2015). The dust discharged into the environment through pulverization and screening for sizes of mineral causes land pollution with heavy metals (Dudka and Adriano, 1977). Continual loading of pollutants into the environment is of great concern for contaminants that are either heavy metal and/or persist in the environment because of their chemical structure (Ali *et al*., 2013; Hashem *et al*., 2017). Such contaminants include lead, cadmium, mercury and dioxin that never go away or degrade for extremely long time. Over a long period of time, a large fraction of these contaminants may become buried in sediments and even small residual amounts of these contaminants are a concern (Sakan and Dordevic, 2010). A persistent bio-accumulative toxin like mercury accumulated and concentrates in the food chain that results to human and wildlife exposure to methyl-mercury (Keating, 2001). Sun (2004) and Zheng *et al*. (1996) reported the chronic effect of arsenic poisoning in several thousands of people living in Ghuizou Province of China and those affected exhibited symptoms of arsenic poisoning such as hyper pigmentation (flush appearance, freckles), Bowen's disease (dark, horny, precancerous lesions of the skin), hyperkeratosis (scaly lesions on the skin, generally concentrated on the hands and feet) and squamous cell carcinoma.

Coal contains a significant amount of pyrites and the exposure of pyrite to atmospheric oxygen through mining activities brings about oxidation of pyrite into ferrous sulphate and sulphuric acid in the presence of bacteria (Ogbonna *et al*., 2018a). The sulphuric acid lowers the pH of soil and water thereby affecting the population and activity of soil organisms inhabiting that environment (Sarma, 2002). Destruction of the vegetal cover during mining operation is accompanied by poorly mixed infertile overburdens, which are generally hostile to soil organisms, thus, mining disturbs the ecosystem by altering the relationship of organisms and their habitat in time and space (Goretti, 1998).

Mesofauna such as centipedes (*Scolopendra cingulata*, Class: Chilopoda, Phylum: Aethropoda) and earthworms (*Hyperiodrilus africanus*, Class: Oligochaeta, Phylum: Annelida) are part of soil fauna community. Dynes (2003) and Vielma-Rondon *et al*. (2003) had shown that not only could earthworm serve as a rich protein source but also as a source of essential amino acids, especially lysine which is limiting in many basic food stuffs and that the amino acid composition of earthworm meal is very similar and potentially superior to that of fishmeal and meat meal. Meat meal are feed ration or meals produced from the meat of animal such as cow. The drive of animal farmers worldwide is to source for cheaper nutritive dietary supplement with high macro and micro-nutrients that may replace fishmeal wholly or partly in animal diet. Earthworm is likely the promising replacement for fishmeal since the value of different species of earthworm as supplement in animal dietary formulation had been rated high (Dedeke *et al*., 2010). Soil macro fauna such as earthworms and centipedes can be used as an indicator or bio-monitor of human impact on ecosystems since the reactions of indicator species to increased concentrations of toxic substances in the laboratory and in the field can be often used to estimate critical levels for soil pollution (Zaitsev and van Straalen, 2001; Skubala and Kafel, 2004).

Literature search show that no work has been carried out on heavy metal and macronutrient in *Hyperiodrilus africanus* and *Scolopendra cingulata* at any coal mine site the world over. It therefore became necessary to establish the heavy metal and macronutrient profile of earthworm commonly found in south east Nigeria. This study, therefore, is aimed at establishing the heavy metal and macro mineral profile of earthworm and centipede species at coal mining sites in Enugu State, Nigeria.

2. MATERIALS AND METHODS

2.1. The Study Area

This study was carried out at Akwuke and Iva mining sites in Coal City, Enugu State, Nigeria. Enugu with an estimated population of 722, 000 in 2006 (NPC, 2006) has very large deposits of sub-bituminous coal, estimated at 1.5 million tons that have been mined since 1916 (Diala, 1984; Ogbonna *et al.*, 2012). It lies within latitude 6° 23' and 6° 26' N and longitude 7° 27' and 7° 30'E (Ogbonna *et al.*, 2018b) and the mean monthly temperature lies between 27 and 29 °C (Ekere and Ukoha, 2013). The area has tropical climate and experiences two seasons both of which are warm. The wet season begins from April and ends October while the dry season begins from November and ends March (Ogbonna *et al.*, 2012). The natural vegetation is tropical rain forest type but has significantly changed over time to guinea savannah due to human activities such as farming (Ezeigbo and Ezeanyim, 1993), nomadism and exploitation of fuel wood, erosion menace, urbanization and rapid increase in human population (Ogbonna *et al.*, 2012). The major streams/rivers in Enugu include the Ekulu, Ogbete and Nyaba rivers. Most streams in the area are not perennial but dry up during the late part of the dry season. Some perennial streams rise from the middle levels of the escarpment near the base of the Ajali sandstone. The streams or rivers, some of which appear fracture-controlled in their flow paths give rise to dendritic drainage pattern (Egboka, 1985). The three largely conformable geologic formations are Enugu shale (Campanian), the Mamu Formation (Lower Maastrichtian) and the Ajali sandstone (Upper Maastrichtian) which constitutes the geology of the Enugu coal mine area. The Enugu shale outcrops occur in the plains east of the north-south trending escarpment. This formation consists of soft grey to dark grey shale and mudstone as well as intercalations of sandstone and sandy shale. The shale’s weather rapidly to red clay soil that forms lateritic capping of considerably thickness (Ezigbo and Ezeanyim, 1993). The Mamu Formation consists of fine to medium grained sandstones, sandy shales, shale’s and mudstones. The formations are highly fractured and are about 395 km in the area and contain pyrite flakes and show sulphur stains (Ezigbo and Ezeanyim, 1993). The Ajali Sandstone which overlies the Mamu Formation consists of thick friable, poorly sorted highly cross-bedded sandstone that is generally white in colour but sometimes is iron stained, and is about 406 m thick in the area that is overlain by lateritic/red earth deposit (Ezigbo and Ezeanyim, 1993).

2.2. Sample Collection and Analysis

Prior to the sample collection, a reconnaissance survey was carried out to determine the altitude of the mined sites (251 and 259 m for Akwuke and Iva mines, respectively) and soil fauna that were common at the 2 mined sites. Two different soil fauna viz *Hyperiodrilus africanus* (earthworm, Family- Eudrilidae) and *Scolopendra cingulata* (Megarian banded centipede, Family- Scolopendridae) were collected from the 2 mined (Akwuke and Iva) and unmined (control) sites in the months of February for dry season and June for wet season. Earthworm and centipede were chosen because they were observed to exist in both mined sites during the reconnaissance survey. Earthworms and centipedes were randomly collected by digging the soil with spade at five 5 m x 5 m line quadrat in four cardinal points (i.e. one sampling point each at north (N), south (S), east (E), west (W), and at the center (C) of each of the two mined sites (Akwuke and Iva). Handsorting of the soils for earthworm was carried out while centipedes were sorted out with hand trowel and cutlass at the sites. The control sample was collected in a 5 year upland bush fallow about 2 km from the mined sites where there was no visible source of contamination. Ten individual samples each of earthworm and centipede were collected from the 2 mined (Akwuke and Iva) and unmined sites (control) and samples from each site were separately transferred in glass containers filled with soil (from each site), covered, well-labeled, placed in a wooden box and covered to avoid contamination from external sources and taken to the laboratory for identification vis-à-vis pre-treatment and analysis. Thereafter, the samples of earthworms and centipedes from N, S, E, W, and C were killed separately, and each sample species (e.g. earthworms) were separately bulked together to give a composite sample. They were then transferred to carefully labeled envelops and oven-dried at 60 ^{o}C for 48 h. The dried samples of each soil fauna were ground into fine particles in an agate mortar. The purpose of collecting the soil fauna was to determine the concentrations of lead (Pb), cadmium (Cd), arsenic (As), iron (Fe), and nickel (Ni) as well as the macro element like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) in the organisms.

To 1 g of each sample was added 10 mL of nitric acid (HNO_3) and 3 mL perchloric acid ($HClO_4$) and the solution was heated until boiling. The sample solution was obtained by processing the residue with hot hydroxyethylcellulose (4 mL), then filtered and diluted with water. The concentrations of Pb, Cd, As, Fe, and Ni in the digested samples were determined using flame Atomic Absorption Spectrophotometer (AAS) (UNICAM 919 model). Triplicate digestion of each sample was carried out and blanks were prepared from only reagents without sample to check for background contamination by the reagents. Appropriate quality assurance procedures and precautions were taken to ensure the reliability of the results. Samples were carefully handled to avoid cross-contamination. Glassware was properly cleaned, and reagents used were of analytical grades. Deionized water was used throughout the study. Working standard solutions of Pb, Cd, As, Fe, and Ni were prepared from the stock standard solutions containing 1000 ppm of element in 2N nitric acid.

For macro-element determination, milled samples of the soil fauna were digested according to the wet digestion method of Novozamsky *et al.* (1983). Calcium and Mg in the digest were determined by EDTA titration method, K was determined by flame photometry, P was determined by the Vanodo-molybdate spectrophotometric method while N was determined by the micro Kjeldahl distillation method (Bremner and Mulvancy, 1982).

2.3. Experimental Design and Data Analysis

A single factor experiment was conducted in a randomized complete block design (RCBD) with 3 replications. Data collected on soil fauna were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 15 and means were separated at $p < 0.05$ using Duncan new multiple range test (DNMRT).

3. RESULTS AND DISCUSSION

3.1. Heavy Metals of Soil Fauna

The concentrations of heavy metals of soil fauna (earthworm and centipede) sampled from Akwuke and Iva mines during the wet and dry season are presented in Tables 1 and 2 respectively. The results show that the highest and the lowest metal concentrations of soil fauna were obtained at the mining sites (Akwuke and Iva) and the control site respectively. The high concentrations of heavy metal in soil fauna at the mined sites may be attributed to mining operations that generated spoil heap and tailings dumped at the sites. Spoil heap and tailings from excavations at mine sites contain heavy metals that contaminate soil and soil organisms at mine sites (Mahboob, 2001). The results also indicate that the concentrations of heavy metals in soil fauna were higher in wet season than in dry season. Flushes of dissolved ions in wet season runoff (acid mine drainage) trigger metal levels in soils, which may bring about metal contamination of soil fauna via dermal contacts (William and Melack, 1991). The concentration of all metals in soil fauna were raised to different degrees (levels) in wet and dry season at the mined sites.

Table 1: Heavy metal concentration (mg/kg) of soil fauna in wet and dry season at Akwuke mine site

	Ni	Pb	Cd	Fe	As
Earthworm wet	$10.10^{a} \pm 0.71$	$8.04^{a} \pm 0.07$	$0.03^{b} \pm 0.01$	$396.00^{a} \pm 1.56$	$0.001^{b} \pm 0.000$
Earthworm dry	$8.62^{ab} \pm 0.88$	$2.00^{b} \pm 0.28$	$0.00^{b} \pm 0.00$	$287.20^{b} \pm 1.70$	$0.000^{b} \pm 0.000$
Earthworm control wet	$0.17^{d} \pm 0.03$	$0.01^{c} \pm 0.01$	$0.00^{b} \pm 0.00$	$9.33^{e} \pm 0.58$	$0.000^{b} \pm 0.000$
Earthworm control dry	$0.10^{d} \pm 0.01$	$0.00^{d} \pm 0.01$	$0.00^{b} \pm 0.00$	$6.00^{f} \pm 1.00$	$0.000^{b} \pm 0.000$
Centipede wet	$6.20^{b} \pm 0.14$	$2.01^{b} \pm 0.04$	$0.12^{a} \pm 0.08$	$208.00^{c} \pm 2.40$	$0.003^{a} \pm 0.001$
Centipede dry	$5.48^{c} \pm 6.11$	$0.84^{c} \pm 0.06$	$0.02^{b} \pm 0.01$	$122.80^{d} \pm 3.96$	$0.000^{b} \pm 0.000$
Centipede control wet	$0.03_{d} \pm 0.02$	$0.00^{d} \pm 0.00$	$0.00^{b} \pm 0.00$	$1.37^{g} \pm 0.12$	$0.000^{b} \pm 0.000$
Centipede control dry	$0.02^{d} \pm 0.01$	$0.00^{d} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.87^{g} \pm 0.25$	$0.000^{b} \pm 0.000$

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p < 0.05$

Table 2: Heavy metal concentration (mg/kg) of soil fauna in wet and dry season at Iva mine site

	Ni	Pb	Cd	Fe	As
Earthworm wet	$18.94^{a} \pm 0.74$	$13.01^{a} \pm 0.07$	$0.01^{b} \pm 0.00$	$580.00^{a} \pm 1.41$	$0.003^{b} \pm 0.001$
Earthworm dry	$7.16^{c} \pm 0.25$	$3.20^{c} \pm 0.27$	$0.00^{b} \pm 0.00$	$247.00^{c} \pm 1.41$	$0.000^{b} \pm 0.000$
Earthworm control wet	$0.17^{e} \pm 0.03$	$0.01^{e} \pm 0.01$	$0.00^{b} \pm 0.00$	$9.23^{e} \pm 0.68$	$0.000^{b} \pm 0.000$
Earthworm control dry	$0.10^{e} \pm 0.01$	$0.00^{e} \pm 0.01$	$0.00^{b} \pm 0.00$	$6.00^{f} \pm 1.00$	$0.000^{b} \pm 0.000$
Centipede wet	$10.74^{b} \pm 0.62$	$6.44^{b} \pm 0.30$	$0.22^{a} \pm 0.04$	$272.90^{b} \pm 2.69$	$0.005^{a} \pm 0.001$
Centipede dry	$4.20^{d} \pm 0.13$	$1.73^{d} \pm 0.06$	$0.00^{b} \pm 0.00$	$151.20^{d} \pm 3.11$	$0.002^{b} \pm 0.014$
Centipede control wet	$0.03^{e} \pm 0.02$	$0.00^{e} \pm 0.00$	$0.00^{b} \pm 0.00$	$1.37^{g} \pm 0.12$	$0.000^{b} \pm 0.000$
Centipede control dry	$0.02^{e} \pm 0.01$	$0.00^{e} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.87^{g} \pm 0.25$	$0.000^{b} \pm 0.000$

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p < 0.05$

Among the metals investigated in soil fauna at the two mined sites, Fe (a trace metal) recorded the highest concentration and this was observed in earthworm (580.00 ± 1.41 mg/kg) during wet season at Iva mine (Table 2), which is significantly ($p<0.05$) higher than the concentrations obtained for Ni, Pb, Cd, and As in earthworm and centipede at the mining and control sites. The concentrations of Fe were 93.65, 95.61, 99.92, and 100 as well as 96.78, 97.79, 100, and 100% higher than the concentrations of Ni, Pb, Cd and As at the mined and control sites, respectively. The results also indicate that the highest concentrations of Ni (18.94 ± 0.74 mg/kg), Pb (13.01 ± 0.07 mg/kg), and Fe (580.00 ± 1.41 mg/kg) in soil fauna was recorded in earthworm during wet season at Iva mine (Table 2), and these values are significantly ($p<0.05$) higher than the highest values obtained for Ni (10.74 ± 0.62 mg/kg) Pb (6.44 ± 0.30 mg/kg), and Fe (272.90 ± 2.69 mg/kg) in centipede during wet season at Iva mine (Table 2). The high level of Ni and Pb in soil at Iva mining site was as a result of tailings and coal processing activities that released these metals (Ni and Pb) into the soil. Thus, the high concentrations of Ni and Pb in earthworm sampled from Iva mine may be attributed to the soil since Ni (79.00 mg/kg) and Pb (81.60 mg/kg) in soils at Iva mine (Table 3) were significantly ($P<0.05$) higher than 19.81 and 21.10 mg/kg recorded for Ni and Pb in soil at Akwuke mine (Table 4), respectively. Consequently, the high concentrations of Ni and Pb in earthworm can be attributed to increase ingestion (consumption) of organic material during wet season vis-à-vis dermal contamination of earthworm by the polluted soils at Iva mine.

The highest concentrations of Cd (0.22 ± 0.04 mg/kg) and As (0.005 ± 0.001 mg/kg) were recorded in centipede during wet season at Iva mine (Table 2) and these values were significantly ($p<0.05$) higher than the highest values recorded for Cd (0.03 ± 0.01 mg/kg) and As (0.003 ± 0.001 mg/kg) in earthworm in this study. The high concentrations of Cd in centipede at Iva mine is also attributed to dermal contact with the soils of Iva mine since Cd (5.02 mg/kg) in soil at Iva mine (Table 3) was significantly ($P<0.05$) higher than 3.06 mg/kg in soil recorded for Cd at Akwuke mine (Table 4). However, the high concentration of As in centipede may be associated with ingestion of prey contaminated with this metal specie (As) at Iva mine. Ni concentration in soil fauna ranged from 5.48 ± 6.11-10.10 ± 0.71 mg/kg at Akwuke mine (Table 1) in wet and dry season which is lower than 4.20 ± 0.13-18.94 ± 0.74 mg/kg recorded at Iva mine (Table 2); Pb ranged from 0.84 ± 0.06-8.04 ± 0.07 mg/kg at Akwuke mine (Table 1) in wet and dry season, which is lower than 1.73 ± 0.06-13.01 ± 0.07 mg/kg recorded at Iva mine (Table 2); Fe ranged from 122.80 ± 3.96-396.00 ± 1.56 mg/kg at Akwuke mine (Table 1) in wet and dry season which is lower than 151.20 ± 3.11-580.00 ± 1.41 mg/kg at Iva mine (Table 2); Cd ranged from 0.03 ± 0.01-0.12 ± 0.08 mg/kg at Akwuke mine (Table 1) in wet and dry season, which is lower than 0.01 ± 0.00-0.22 ± 0.04 mg/kg recorded at Iva mine (Table 2) while As ranged from 0.001 ± 0.000-0.003 ± 0.001 mg/kg at Akwuke mine (Table 1) in wet and dry season, which is lower than 0.002 ± 0.014-0.005 ± 0.001 mg/kg at Iva mine (Table 2). Thus, the contamination of soil fauna by the metals show that earthworm and centipede can be used as environmental indicators for monitoring metal polluted sites. Heavy metal contamination of soils in the study area may result in the contamination of soil fauna such as earthworms. Indeed, earthworms are important bait in fishing, *inter alia*, food material for fish production in south-eastern Nigeria (Ogbonna *et al*., 2013). From ecological perspective, assessment of metal accumulation in earthworms is important since they are important prey to many amphibian, reptile, bird, and mammalian species (OECD, 2004). Thus, metal accumulation may increase along the food chain due to bioaccumulation (Ameh *et al*., 2012) and bio-magnifications in biota. Fish, a highly nutritious food with known benefits to mankind, is the main route of human exposure to heavy metals (Ogbonna *et al*., 2013). High levels of Hg exposure have been reported in numerous fish-eating populations throughout the world (Pirrone and Mahaffey, 2005). The majority of these people work or reside near oceans, major lakes and rivers, or hydroelectric dams, and are often dependent on local catch, with fish an integral part of their culture and traditions (Mergler *et al*., 2007). Nickel (Ni) is essential in animal nutrition and significant level of Ni is present in Ribonucleic acid (RNA) and Deoxy-nucleic acid (DNA) (Di Ferrante, 1979). However, Dara (1993) established the crucial role of Ni to some microorganisms and animals by its interference with heme-synthesis and leading to hematological damage and also impairs the activity of porphobilinogen decarboxylase.

3.2. Macronutrient Content of Soil Fauna

The macronutrient content of soil fauna during wet and dry season at Akwuke and Iva mine are presented in Tables 5 and 6, respectively. The results indicate that the highest and the lowest macronutrient of soil fauna was recorded at the mined and unmined (control) sites, respectively. Ameh *et al.* (2012) and Olayinka *et al.* (2011) in their study on earthworm as bio-indicator of oil contaminated soil and cement factory site in Nigeria reported higher content of macro (Ca, P, and K) and micro (Zn, Mn, and Fe) elements in earthworm obtained from metal polluted soil than at the control sites, respectively. The highest content of Ca (6.01 ± 0.04 cmol/kg), Mg (190.60 ± 2.83 cmol/kg), K (150.00 ± 2.26 cmol/kg), and N (1.28 ± 0.18 cmol/kg) were recorded in earthworms obtained at Akwuke mine and these values are significantly ($P<0.05$) higher than the highest corresponding values obtained for soil fauna at Iva mine (2.54 ± 0.07, 87.00 ± 2.40, 108.20 ± 2.69, and 0.57 ± 0.08 cmol/kg) and the unmined site (2.60 ± 0.10, 73.83 ± 2.12, 57.10 ± 1.95, and 0.61 ± 0.03 cmol/kg). The content of Ca, Mg, K, and N obtained from earthworm at Akwuke mine were 22.78, 24.76, 34.32 and 23.17% as well as 23.32, 21.01, 18.11 and 24.80% higher than the values obtained from earthworms and centipedes at Iva mine and the unmined site, respectively. The low level of heavy metal (Ni, Pb, and Cd) contamination in soil at Akwuke mine (Table 4) is implicated for the high content of Ca, Mg, K, and N in earthworm obtained from Akwuke mine (unlike Iva mine and the unmined site with high metal contamination and low contamination levels of metals, respectively (Table 3).

Table 3: Heavy metal concentration (mg/kg) in soil at Iva mine in wet and dry season (Ogbonna et al., 2018b)

Location	Depth	Season	Ni	Pb	As	Fe	Cd
Crest	0-10cm	Wet	28.01^{g} ± 0.03	55.70^{d} ± 1.13	0.00 ± 0.00	377.30^{j} ± 3.82	5.02^{a} ± 0.11
		Dry	20.05^{i} ± 0.21	48.25^{e} ± 0.21	0.00 ± 0.00	353.00^{k} ± 1.27	0.11^{kl} ± 0.03
	10-20cm	Wet	15.25^{j} ± 0.42	45.30^{f} ± 0.57	0.00 ± 0.00	387.50^{i} ± 3.54	2.87^{c} ± 0.13
		Dry	10.05^{k} ± 0.78	22.05^{m} ± 0.35	0.00 ± 0.00	238.60^{n} ± 0.28	0.07^{kl} ± 0.03
	20-30cm	Wet	10.00^{k} ± 0.57	38.10^{h} ± 0.28	0.00 ± 0.00	402.80^{fg} ± 2.55	1.01^{g} ± 0.13
		Dry	6.10^{l} ± 0.42	29.40^{j} ± 0.85	0.00 ± 0.00	218.00^{o} ± 2.83	0.48^{ij} ± 0.13
Middle slope	0-10cm	Wet	46.45^{d} ± 0.16	37.50^{hi} ± 0.28	0.00 ± 0.00	410.30^{e} ± 2.40	0.49^{ij} ± 0.08
		Dry	21.05^{i} ± 1.34	30.01^{j} ± 1.40	0.00 ± 0.00	238.60^{n} ± 1.56	0.19^{k} ± 0.01
	10-20cm	Wet	64.12^{b} ± 0.35	65.04^{c} ± 0.34	0.00 ± 0.00	467.80^{c} ± 3.11	1.64^{e} ± 0.07
		Dry	40.06^{e} ± 1.33	43.60^{g} ± 1.56	0.00 ± 0.00	392.70^{hi} ± 3.82	0.04^{kl} ± 0.03
	20-30cm	Wet	79.00^{a} ± 0.57	81.60^{a} ± 0.57	0.00 ± 0.00	498.20^{a} ± 2.55	1.86^{d} ± 0.23
		Dry	52.30^{c} ± 0.57	69.70^{b} ± 2.40	0.00 ± 0.00	408.00^{ef} ± 2.83	1.17^{f} ± 0.04
Valley	0-10cm	Wet	15.20^{j} ± 0.57	36.65^{hi} ± 0.35	0.00 ± 0.00	328.50^{l} ± 2.12	3.08^{b} ± 0.03
		Dry	10.06^{k} ± 0.65	23.85^{l} ± 0.21	0.00 ± 0.00	397.70^{gh} ± 0.42	0.01^{kl} ± 0.01
	10-20cm	Wet	23.50^{h} ± 0.37	35.90^{i} ± 0.85	0.00 ± 0.00	454.70^{d} ± 0.42	0.74^{h} ± 0.07
		Dry	10.05^{k} ± 0.49	27.01^{k} ± 0.01	0.00 ± 0.00	273.70^{m} ± 0.42	0.08^{kl} ± 0.03
	20-30cm	Wet	31.07^{f} ± 0.38	26.10^{k} ± 0.28	0.00 ± 0.00	490.00^{b} ± 5.66	0.61^{hi} ± 0.04
		Dry	23.13^{h} ± 0.33	19.60^{n} ± 0.28	0.00 ± 0.00	381.40^{j} ± 1.98	0.41^{j} ± 0.07
Control	0-10cm	Wet	0.02^{m} ± 0.01	1.48^{o} ± 0.12	0.00 ± 0.00	70.60^{r} ± 0.57	0.01^{kl} ± 0.00
		Dry	0.02^{m} ± 0.01	0.72^{o} ± 0.04	0.00 ± 0.00	43.00^{t} ± 0.57	0.00^{l} ± 0.00
	10-20cm	Wet	0.01^{m} ± 0.00	1.01^{o} ± 0.16	0.00 ± 0.00	82.00^{q} ± 0.71	0.00^{l} ± 0.00
		Dry	0.00^{m} ± 0.00	0.40^{o} ± 0.06	0.00 ± 0.00	38.50^{t} ± 0.85	0.00^{l} ± 0.00
	20-30cm	Wet	0.01^{m} ± 0.01	0.26^{o} ± 0.10	0.00 ± 0.00	101.00^{p} ± 1.41	0.00^{l} ± 0.00
		Dry	0.00^{m} ± 0.00	0.22^{o} ± 0.08	0.00 ± 0.00	62.00^{s} ± 4.24	0.00^{l} ± 0.00

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p < 0.05$

Calabrese and Baldwin (2003) showed that low concentration of toxicants can have positive effects on soil organisms. They explained that litter consumption rate per biomass increases at low pollution levels. Lukkari *et al.* (2004) and Morgan *et al.* (2004) showed that levels of a number of regulatory proteins were increased in field earthworms exposed to metal pollution. This protein production could increase energy demand and they explained that at low pollution levels more energy is needed to regulate and detoxify heavy metals, resulting in higher litter consumption (Hobbelen *et al.*, 2006).

Indeed, the content of all the macronutrient in soil fauna (earthworms and centipedes) were raised to different levels in wet and dry season at the mined sites. The highest content of Ca, Mg, K and N were observed in earthworms at Akwuke mine during wet season (Table 5) and this may be attributed to increase activities of this soil fauna (i.e. breakdown and ingestion of organic material) during wet season. Earthworms constitute a major component in soil functioning, and they play an important role in chemical element transformations (Lee, 1985; Lavelle *et al.*, 2004). However, the value of P (3.04 ± 0.08 cmol/kg) in centipede during wet season at Akwuke mine was higher than the highest value of phosphorus in earthworm (1.62 ± 0.04 cmol/kg). The value of P in centipede is 30.47% higher than its (P) value in earthworm. Generally, the values of the macro elements content of soil fauna followed a decreasing order: Mg>K>Ca>P>N.

Table 4: Heavy metal concentration (mg/kg) in soil at Akwuke mine site in wet and dry season (Ogbonna et al., 2018a)

Location	Depth (cm)	Season	Ni	Pb	As	Fe	Cd
Crest	0-10	Rainy	$9.71^{efg} \pm 0.16$	$15.45^{bc} \pm 0.18$	0.00 ± 0.00	$417.00^{f} \pm 1.98$	$1.73^{c} \pm 0.06$
		Dry	$4.15^{k} \pm 0.07$	$12.65^{cd} \pm 0.13$	0.00 ± 0.00	$296.10^{k} \pm 8.63$	$0.68^{de} \pm 0.07$
	10-20	Rainy	$11.72^{d} \pm 0.07$	$15.80^{bc} \pm 0.28$	0.00 ± 0.00	$480.70^{c} \pm 2.40$	$0.72^{d} \pm 0.17$
		Dry	$10.04^{ef} \pm 0.20$	$10.45^{de} \pm 0.07$	0.00 ± 0.00	$290.00^{k} \pm 4.95$	$0.07^{gh} \pm 0.03$
	20-30	Rainy	$14.30^{c} \pm 0.71$	$17.62^{b} \pm 0.08$	0.00 ± 0.00	$522.00^{a} \pm 1.98$	$0.61^{de} \pm 0.13$
		Dry	$8.24^{hi} \pm 0.20$	$13.60^{cd} \pm 0.28$	0.00 ± 0.00	$216.40^{n} \pm 1.27$	$0.38^{f} \pm 0.03$
Middle slope	0-10	Rainy	$10.45^{e} \pm 0.27$	$15.10^{bc} \pm 0.28$	0.00 ± 0.00	$428.10^{e} \pm 0.85$	$2.92^{b} \pm 0.03$
		Dry	$6.04^{j} \pm 0.06$	$10.41^{de} \pm 0.11$	0.00 ± 0.00	$362.20^{i} \pm 4.53$	$0.10^{gh} \pm 0.04$
	10-20	Rainy	$11.65^{d} \pm 0.11$	$12.60^{cd} \pm 0.35$	0.00 ± 0.00	$458.00^{d} \pm 2.12$	$0.17^{g} \pm 0.06$
		Dry	$8.04^{i} \pm 0.20$	$8.21^{ef} \pm 0.44$	0.00 ± 0.00	$267.60^{l} \pm 1.56$	$0.01^{h} \pm 0.01$
	20-30	Rainy	$12.02^{d} \pm 0.48$	$10.30^{de} \pm 0.57$	0.00 ± 0.00	$506.70^{b} \pm 0.42$	$0.08^{gh} \pm 0.01$
		Dry	$9.20^{fgh} \pm 0.42$	$6.11^{f} \pm 0.13$	0.00 ± 0.00	$314.00^{j} \pm 1.56$	$0.02^{h} \pm 0.01$
Valley	0-10	Rainy	$8.85^{ghi} \pm 0.35$	$15.26^{bc} \pm 6.87$	0.00 ± 0.00	$397.80^{g} \pm 11.03$	$3.06^{a} \pm 0.08$
		Dry	$6.07^{j} \pm 0.10$	$17.50^{b} \pm 0.42$	0.00 ± 0.00	$389.80^{h} \pm 0.28$	$0.08^{gh} \pm 0.03$
	10-20	Rainy	$16.03^{b} \pm 0.21$	$21.10^{a} \pm 0.85$	0.00 ± 0.00	$258.70^{m} \pm 0.42$	$0.57^{e} \pm 0.10$
		Dry	$10.04^{ef} \pm 1.47$	$15.65^{bc} \pm 0.35$	0.00 ± 0.00	$207.80^{o} \pm 3.11$	$0.05^{gh} \pm 0.03$
	20-30	Rainy	$19.81^{a} \pm 1.29$	$21.72^{a} \pm 0.69$	0.00 ± 0.00	$203.00^{o} \pm 4.24$	$0.29^{f} \pm 0.01$
		Dry	$12.01^{d} \pm 0.16$	$13.28^{cd} \pm 0.11$	0.00 ± 0.00	$186.00^{p} \pm 2.83$	$0.11^{gh} \pm 0.03$
Control	0-10	Rainy	$0.02^{l} \pm 0.00$	$1.46^{g} \pm 0.23$	0.00 ± 0.00	$70.60^{s} \pm 1.98$	$0.01^{h} \pm 0.00$
		Dry	$0.02^{l} \pm 0.00$	$0.72^{g} \pm 0.04$	0.00 ± 0.00	$43.00^{u} \pm 0.99$	$0.00^{h} \pm 0.00$
	10-20	Rainy	$0.01^{l} \pm 0.00$	$1.01^{g} \pm 0.16$	0.00 ± 0.00	$82.00^{r} \pm 2.83$	$0.00^{h} \pm 0.00$
		Dry	$0.00^{l} \pm 0.00$	$0.40^{g} \pm 0.04$	0.00 ± 0.00	$38.50^{u} \pm 0.99$	$0.00^{h} \pm 0.00$
	20-30	Rainy	$0.01^{l} \pm 0.01$	$0.26^{g} \pm 0.04$	0.00 ± 0.00	$101.00^{q} \pm 4.24$	$0.00^{h} \pm 0.00$
		Dry	$0.00^{l} \pm 0.00$	$0.22^{g} \pm 0.14$	0.00 ± 0.00	$62.20^{t} \pm 1.41$	$0.00^{h} \pm 0.00$

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p < 0.05$

Table 5: Macronutrient content (cmol/kg) in soil fauna in wet and dry seasons at Akwuke mine site

	Ca	Mg	K	N	P
Earthworm wet	$6.01^{a} \pm 0.04$	$190.60^{a} \pm 2.83$	$150.00^{a} \pm 2.26$	$1.28^{a} \pm 0.18$	$1.62^{d} \pm 0.04$
Earthworm dry	$2.01^{de} \pm 0.10$	$130.30^{b} \pm 1.70$	$127.80^{b} \pm 2.40$	$0.72^{b} \pm 0.06$	$1.03^{f} \pm 0.06$
Earthworm control wet	$2.60^{c} \pm 0.10$	$73.83^{c} \pm 2.12$	$57.10^{e} \pm 1.95$	$0.61^{bc} \pm 0.03$	$1.92^{c} \pm 0.26$
Earthworm control dry	$1.71^{d} \pm 0.11$	$68.67^{de} \pm 1.53$	$53.87^{e} \pm 1.63$	$0.48^{bcd} \pm 0.06$	$1.42^{de} \pm 0.06$
Centipede wet	$4.41^{b} \pm 0.41$	$70.40^{cd} \pm 1.84$	$86.40^{d} \pm 1.98$	$0.22^{d} \pm 0.11$	$3.04^{a} \pm 0.08$
Centipede dry	$2.06^{d} \pm 0.08$	$62.00^{f} \pm 2.40$	$104.10^{c} \pm 1.41$	$0.29^{d} \pm 0.11$	$2.26^{b} \pm 0.17$
Centipede control wet	$2.23^{d} \pm 0.02$	$67.33^{de} \pm 1.15$	$55.33^{e} \pm 3.50$	$0.47^{bcd} \pm 0.20$	$1.30^{ef} \pm 0.09$
Centipede control dry	$2.07^{d} \pm 0.19$	$64.67^{ef} \pm 2.52$	$52.00^{e} \pm 4.00$	$0.44^{cd} \pm 0.12$	$1.09^{f} \pm 0.07$

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p < 0.05$

Table 6: Macronutrient content (cmol/kg) in soil fauna in wet and dry seasons at Iva mine site (Ogbonna et al., 2018b)

	Ca	Mg	K	N	P
Earthworm wet	$0.18^{d} \pm 0.03$	$39.20^{d} \pm 0.28$	$87.10^{b} \pm 0.99$	$0.57^{a} \pm 0.08$	$1.12^{c} \pm 0.16$
Earthworm dry	$0.08^{d} \pm 0.04$	$87.00^{a} \pm 2.40$	$108.20^{a} \pm 2.69$	$0.13^{c} \pm 0.06$	$0.81^{d} \pm 0.06$
Earthworm control wet	$2.60^{a} \pm 0.10$	$73.83^{b} \pm 2.12$	$57.10^{d} \pm 1.95$	$0.61^{a} \pm 0.03$	$1.92^{a} \pm 0.26$
Earthworm control dry	$1.71^{c} \pm 0.11$	$68.67^{c} \pm 1.53$	$53.87^{d} \pm 1.63$	$0.48^{a} \pm 0.06$	$1.42^{b} \pm 0.06$
Centipede wet	$2.54^{a} \pm 0.07$	$76.00^{b} \pm 2.69$	$53.30^{d} \pm 2.40$	$0.38^{ab} \pm 0.10$	$0.64^{d} \pm 0.07$
Centipede dry	$1.62^{c} \pm 0.11$	$41.30^{d} \pm 2.40$	$80.00^{c} \pm 1.98$	$0.20^{bc} \pm 0.08$	$2.10^{a} \pm 0.16$
Centipede control wet	$2.23^{b} \pm 0.02$	$67.33^{c} \pm 1.15$	$55.33^{d} \pm 3.50$	$0.47^{a} \pm 0.20$	$1.30^{bc} \pm 0.09$
Centipede control dry	$2.07^{b} \pm 0.19$	$64.67^{c} \pm 2.52$	$52.00^{d} \pm 4.00$	$0.44^{a} \pm 0.12$	$1.09^{c} \pm 0.07$

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p < 0.05$. Mean separation was done using the Duncan Multiple Range Test (DMRT)

4. CONCLUSION

The study on determination of heavy metal and macronutrient in *Hyperiodrilus africanus* and *Scolopendra cingulated* showed with certainty that earthworm and centipede accumulated heavy metals and macronutrients at the coal mine sites. Higher levels of Cd and As were accumulated in centipede while Ni, Pb, and Fe were accumulated in earthworm during the wet season at Iva mine than Akwuke mining site, which could result to bioaccumulation and bio-magnifications of these metals along the food chain.

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6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this work.

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