HOLOS ENVIRONMENT

HEAVY METAL BIOMONITORING AND ENTOMOFAUNA DISTRIBUTION OF ENYIGBA MINES IN SOUTHEAST, NIGERIA

BIOMONITORIZAÇÃO DE METAIS PESADOS E ENTOMOFAUNA DISTRIBUIÇÃO DE MINAS DE ENYIGBA NO SUDESTE, NIGÉRIA

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Abstract: Heavy metals are metals with high atomic weight and substances with high electrical conductivity that voluntarily lose their electrons to form cations. The heavy metal biomonitoring and entomofauna distribution of enyigba mines in southeast, Nigeria was studied to determine the contamination of heavy metals base and attendant health risks using standard entomological techniques and atomic absorption spectrophotometer (AAS). Insects examined were *Reticulitermes flavipes, Zonocerus elegans, Acraea acrita, and Crematogaster* sp collected from Royal Salt mining sites. The study revealed the accumulation index of Po4 (683.70±677.50) was high followed by Mn, (11.00 ±10.90) Cu, (7.600 ±6.60) Cd, (0.350±0.145) at $p<0.05$. This is high compared to the codex standards. ANOVA results declared the concentration of heavy metals above permissible limits with a significant difference between site A (SA) and the control site (CS) at ($p<0.0001$). Also, there is an important difference observed between site A (SA) and site B (SB) ($p<0.0001$). The relatively higher concentrations of metals were found in Orthoptera, followed by termite where ants recorded the lowest metal concentration. The study further revealed that remarkable values were recorded in the Control Site (CS) (32944) followed by (SB) (20904) while SA recorded the least value (6644). However, the low species diversity and abundance in different sites is an indication of the impacts of heavy metals accumulation in the sites. However, accumulation of PO_4 in the insects, especially at sites A and B, showed the effects of mining on PO_4 generation is high and could pose health risks to human life if not mitigated. However, the wet season recorded higher abundance compared to the dry season on *Reticulitermes flavipes* followed by *Crematogaster sp.* whereas *Acraea acrita* recorded the least abundance.

Keywords: Heavy metals. Entomofauna. Mining site. Ebonyi State.

Resumo: Metais pesados são metais com alto peso atômico e substâncias com alta condutividade elétrica que perdem voluntariamente seus elétrons para formar cátions. O biomonitoramento de metais pesados e a distribuição da entomofauna das minas de Enyigba no sudeste da Nigéria foram estudados para determinar a contaminação da base de metais pesados e os riscos à saúde associados usando técnicas entomológicas padrão e espectrofotômetro de absorção atômica (AAS). Os insetos examinados foram Reticulitermes flavipes, Zonocerus elegans, Acraea acrita e Crematogaster sp coletados de locais de mineração de sal real. O estudo revelou que o índice de acumulação de Po4 (683,70 ± 677,50) foi alto,

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seguido por Mn, (11,00 ± 10,90) Cu, (7,600 ± 6,60) Cd, (0,350 ± 0,145) em $p < 0,05$. Isso é alto em comparação com os padrões do códice. Os resultados da ANOVA declararam concentração de metais pesados acima dos limites permitidos com uma diferença significativa entre o local A (SA) e o local de controle (CS) em ($p<0,0001$). Além disso, há uma diferença significativa observada entre o local A (SA) e o local B (SB) ($p<0,0001$). As concentrações relativamente maiores de metais foram encontradas em Orthoptera, seguido por cupins, onde a formiga registrou a menor concentração de metais. O estudo revelou ainda que valores notáveis foram registrados no local de controle (CS) (32944), seguido por (SB) (20904), enquanto SA registrou o menor valor (6644). No entanto, a baixa diversidade e abundância de espécies em diferentes locais é uma indicação dos impactos do acúmulo de metais pesados nos locais. No entanto, o acúmulo de PO4 nos insetos, especialmente nos locais A e B, mostrou que os efeitos da mineração na geração de PO4 são altos e podem representar risco à saúde humana se não forem mitigados. No entanto, a estação chuvosa registrou maior abundância em comparação à estação seca em Reticulitermes flavipes, seguido por Crematogaster sp. enquanto Acraea acrita registrou a menor abundância.

Palavras-chave: Metais pesados. Entomofauna. Local de mineração. Estado de Ebonyi.

1 INTRODUTION

Insects (Latin-*insectum*) are groups of biological taxa (invertebrates) of the class Insecta. They are the largest group within the arthropod phylum with diverse behaviors and adaptations (Wilson 2009). Insects are the most diverse group of organisms, including more than a million described species, and represent more than half of all known living organisms). The total number of existing species is estimated at between six and ten million, and over 90 % of the animal life forms on Earth are insects (Erwin, 1997). Insects are used to study water quality and environmental contaminations since they are extremely sensitive to environmental changes capable of disrupting their core functions, change their life cycle such as growth, metabolism, and reproduction (David, 1989). Insects such as butterflies have been extensively used as heavy metal and environmental pollution bioindicators that are close to industrial states and even in the metropolitan regions (Renato *et al*., 2010).

Termites are “ecosystem engineers”, which act as bioindicators of soil fertility through the determination of soil physico-chemical parameters. Ants are ubiquitous in almost all the known trophic levels of the web, food chains, and other ecological functions. They serve as a good bioindicator group for various types of pollution (Kaspari; Majer, 2000; Andersen *et al.,* 2002) sensors soil-heavy metal contamination (Gramigni *et al.,* 2013), aerial phthalate pollution (Lenoir *et al*., 2014) and land rehabilitation. The Red wood ant *Formica lugubris* accumulates heavy metals such as Aluminum (Al), Cadmium (Cd), Cobalt (Co), Copper (Cu), Iron (Fe), Nickel (Ni), Lead (Pb), and Zinc (Zn) both in worker ant bodies and nest material (Skaldina *et al.,* 2018). Ants are increasingly being used as bioindicators to monitor ecosystem health conditions (Akhila and Keshamma, 2022).

Insects deliver a host of ecological services fundamental to the survival of mankind and play an important role in plant reproduction (Ratcliffe, 1970), Insect behaviors and abundance are key contributors to the ecology of insect interactions with plant and animal species, as well as with their abiotic environments, these behaviors are critical to the stability, sustainability and diversity of ecosystems (Fisher, 1998). Ecosystem services are the specific benefits people derive from nature through the interaction between plants, animals, humans and their surroundings such as farms, cities and other creatures (MA (Millennium Assessment), 2005). Plant-insect interactions are quite common in nature and sometimes very useful for both species. However, in terrestrial ecosystems insects play the key quintessential ecological roles in diverse ecological processes such as seed dispersal, nutrient cycling, bioturbation (De Groot, Wilson and Boumans, 2002; Nichols, *et al.*,2008; Fincher, Monson, and Burton 1981). Heavy metal are group of metals and metalloids of relatively high atomic mass (>4.5 g/cm3) such as Pb, Hg, Cu, Cd, Sn, and Zn, capable of causing toxicity problems (Kemp, 1998). Metals are found all over the earth including the atmosphere, earth crust, water bodies, and can also accumulate in biological organisms including plants and animals (Duffus, 2002; Li, 2017). They were incredibly effective in ground surveys due to their high mobility. Insects are used for bioassessment such as monitoring of pesticides, heavy metals, and radionuclides levels (Porrini *et al.,* 2003).

Some of these heavy metals such as cobalt, chromium, copper, magnesium, iron, molybdenum, manganese, selenium, nickel and zinc are essential nutrients that are required for various physiological and biochemical functions in the body and may result to deficiency diseases or syndromes if not in adequate amounts (WHO/FAO/IAEA, 1996), but in large doses they may cause acute or chronic toxicities.

Heavy metal contamination emanates from industrial effluents directly spread to the environment, bioaccumulate in plants thereby causing phytotoxicity problem to plants and likely the most serious environmental and public health concern (Gu *et al.*, 2018; Wang *et al.*, 2020). Natural ecosystems all over the world have been adversely affected by human interventions (Qadir and Malik, 2009) Modern farming, industrialization, and increased vehicular use have led to high concentrations of heavy metals such as lead, nickel, chromium, cadmium, aluminum, mercury, and zinc in the environment (Atafar and Mesdaghinia; Nouri *et al.*, 2010). Plant-insect interactions are quite common in nature and sometimes very useful for both species (Agboka *et al*. 2010). Insect pollinators and flowering plants have mutual relationships (Bezzi *et al*., Arenas and Farina, 2012). Nectar and pollen are food rewards for pollinators (Bezzi *et al*., 2010, Arenas and Farina, 2012). It is obvious that when these heavy metals are ingested through food (plants, animals, insects or water) into the body, they are acidified by the acid medium of the stomach (Chrestensen, 2000).

Interestingly, toxicity occurs along the food chain when contaminated species is consumed by another organism on their higher trophic level (Heng, 2012). When ambient temperature exceeds the upper temperature threshold, the thermal stress sometimes lead to individual insect death and even subject insect to immediate local population extinction in insects (Wang *et al*., 2916a). Furthermore, as average temperature rise, the habitable range for many species is predicted to shift (Honnay, 2002).

1.1 Study Area

Ebonyi State is rich with mineral deposits in Southeast, Nigeria with Salt Lake at Okposi, Uburu and Oshiri, Lead and Zinc at Enyigba and Enyim-Chukwu as well as Kaolin and Limestone at Ishiagu, Afikpo, and Nkalagu. Royal Salt is an important and leading industrial area of Ikwo, Ebonyi State Nigeria, Ebonyi State occupies a landmass of approximately 6,400 km^2. and falls within an area of moderate relief with elevations between 125m and 245m above sea level (Edeani, Ezeh, and Ezike, 2013). Ebonyi State shares boundaries with Cross River State in the East, Benue State in the North, Enugu State in the West, and Abia State in the South (Annual Abstract of Statistics, 2012).

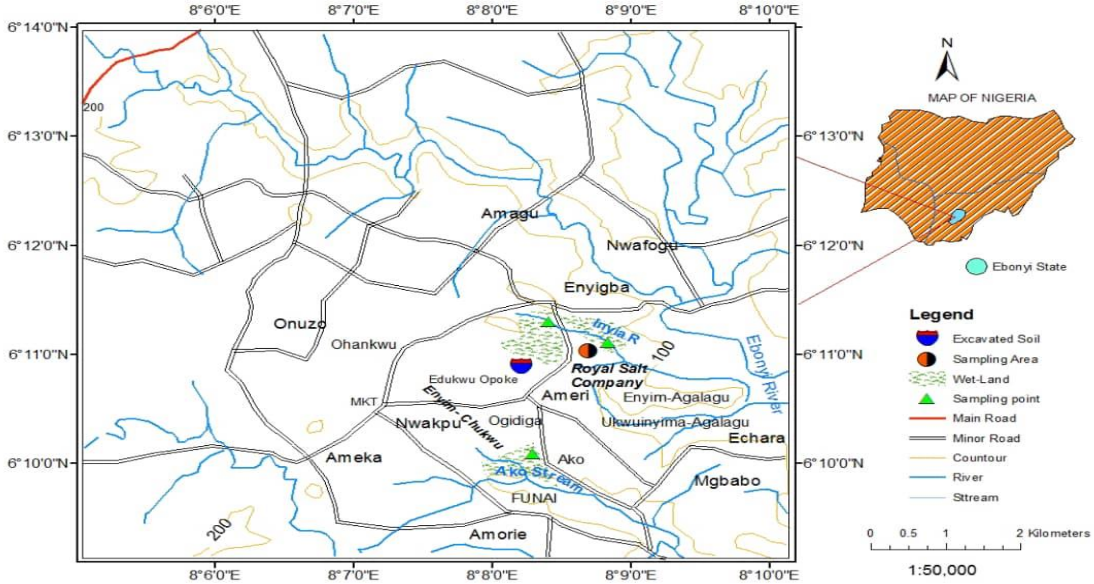
The coordinates of the Royal Salt Mining Site fall within 6°10'49''N, 8°8'24E. For site A; 6° 10'44''N,8°8'30''E for site B; and 6°8'49''N, 8°8'37''E for CONTROL Site and covered land of about 70km^2. The general landform is undulating with dunes, and the area is covered with vast loam and non-marshy soil that favors agricultural practices. The study zone is mostly loamy soil, rich in organic manures. It is predominantly a pre-urban setting with mainly thick trees, palm trees, gmelina trees, neem trees, mango trees, grassy areas, and houses. The landscapes are characterized by several rock formations.

The soil type is generally a dark kind of ferralsols, variation consists of shallow/stony reddish clay at the feet of inselbergs in the higher sections, lateritic clay and fine-grained to sandy soils in the upper slope/lateritic table lands, and ferruginous soils on the crystalline acid rocks of the basement complex. Farming is a predominant occupation in the area irrespective of age and gender. About 80% of the inhabitants are farmers and a few civil servants are also engaged in part-time farming and have formed the major source of livelihood in the area. Basic social amenities are lacking with minimal or poor state of sewage systems in most communities. There is also a very low literacy status dominating the population in the area. Fishing is also carried out on a commercial level, particularly along Ebonyi River and other minor rivers in the area and has been the major occupation of people inhabiting such places. Livestock farming is also common. This includes rearing animals such as goats, chickens, pigs, local cattle, and pets. Mineral and natural resources include; lead (tangele), zinc, limestone, and timbers.

1.2 Study Location

The study area is located in Ikwo in Ebonyi State, South-eastern Nigeria (Fig. 1). Ikwo is situated at the eastern part of the State with a land mass of approximately 500km, and shares borders with Abakaliki, Izzi and Ezza South Local Government Areas. The sampling points include Royal Salt surrounding Farm lands and fallows (wetlands) located in Enyim-Agalagu Ikwo, and Ako surrounding Farm lands and fallows (used as control). There are some other significances of human hamlets dotted the area, which indicated presence of indigenes that engaged in the subsistence farming and other anthropogenic activities close to the site.

Figure 1 - Map of Nigeria showing Ebonyi state and the sampled area



Source: Geology Department, EBSU

2 MATERIALS AND METHODS

The study heavy of heavy metal contamination was carried out at the Royal Salt mining site in three different sites which include: upper Inyia (SA), lower Inyia (SB), and the control site. Four (4) insects' groups were collected for the analysis. It includes *Reticulitermes flavipes, Zonocerus elegans Acraea acrita, Crematogaster sp.* These were collected from Royal Salt mining sites and the control site (AKO). This sample collection was also carried out in the control site (CS) where mining is not nonexistent.

2.1 Heavy metals Analysis of insects

Sample Processing: The collected insect samples were relaxed with hot water and transferred into a sampling bottle after sorting, capped properly, and were immediately taken to the Laboratory for Analysis. On arrival at the Lab, the samples were registered and were mechanically dried in an electric oven set 65^{o}C and milled with Thomans Willey milling machine through a 0.6mm sieve, and kept for further treatment.

2.2 Insect Collection Methods

2.2.1 Use of Sweep net

A sweep net with a mouth diameter of 38cm made of a lightweight quaze with aluminium handle of approximately 100cm m in length was used. The sweep net was swung back and forth through the vegetation folded the net over with the insect at the bottom of the net and then caught/transferred to the killing jar (insect bottle). Certain precautions are adopted for active (stinging insects), such as wasps including other stinging insects in the order Hymenoptera to avoid escaping by folding the insect over with the insect in the bottom of the net, stunning and then picking the insect out of the net and pinned for preservation or put into the bottle, other invertebrates which does not sting (i.e. flying insects fauna such as butterflies, dragonflies, small beetles, and bugs), the catch was removed by tubing individual specimens, picking the insects out of the net and put them into the bottle, or this was achieved by gently holding the net mouth and immediately trace the catches from the mouth of the net to the bottom of the net and any invertebrate captured were put at the killing jar and put in the detachable zip-off net bags for later sorting. The efficiency of sampling was increased by using detachable zip-off net bags, which can be quickly removed and stored for later sorting. The insects caught were identified using Insects of Nigeria - Check List and Bibliography. The identification of the specimens was verified in the Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The voucher specimens were also kept as reference point for further studies.

2.2.2 Pitfall traps

Ten pitfall traps with mouth diameters of 9.80 cm and 6.2 cm deep, made of plastic containers, were sunk in the ground with their rims flush with ground level at the two study sites and the control site, on each sampling occasion (i.e. every month). The traps were filled to one-third with 5 % formalin (Ewuim *et al.*, 2007). The traps were inspected every twenty-four hours and a good number of the surface-living invertebrates in low vegetation and bare ground, particularly larger beetles, ants, and even a few small grasshopper species inadvertently fell into the traps, the insects caught were sorted, identified, and counted.

The insects were identified using Insects of Nigeria - Checklist and Bibliography by Medler (1980). The Insects collected were sent to the Department of Crop Protection, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria with information such as the name of the collector, place of collection, and date of collection for proper taxonomical identification.

The voucher specimens were kept at Nnamdi Azikiwe University Awka as reference point for further studies.

2.2.3 Light Traps

Light traps constructed with the plank, designed with lightweight quaze with 135-W Mercury-Vapor (MV) bulb and baffles positioned inside the box and stationed through the generator were positioned at the two sites including the control site (Waring, 1980). Nocturnal insects such as moths, flies, and other night-flying insects were attracted towards the light source, hit on the baffle, and fall into the trap. The traps were inspected every twenty-four hours, and the insects caught were sorted, identified, and counted under a dissecting microscope. The insects were identified using Insects of Nigeria–Check List and Bibliography by Medler (1980).

The voucher specimens were also kept as reference points for further studies.

2.3 Sample Digestion by the Multiple Nutrients

0.1g (100mg) of the sample was weighed into a 150 ml Pyrex conical flask, 5.0 ml of the multiple nutrient extraction mixture (H_2S0_4-selenium salicylic acid) was added to the sample and allowed to stand for 24 hours (overnight). The sample was placed on a heat-in-digestion block set at 32^oC and allowed to heat for 4 hours under the fume cupboard. Then, 5 ml of conc HCL was added to the sample and the heating temperature increased to 80^{o}c. The sample was allowed to heat vigorously until the appearance of a white perchloric fume appeared and a clear solution was obtained then the digestion terminated. The sample was brought down from the hot plate and allowed to cool and finally transferred into a 50 ml volumetric flask using distilled water to wash down and make up to the mark.

3 AAS ANALYSIS

The digestion was used to determine the various metals using the USA Bulk scientific Atomic Absorption Spectrophotometer (AAS). Descriptive statistics, analysis of variance (one-way) ANOVA, and correction, were carried out on recorded data by testing significance differences at $p<0.05$.

4 RESULTS

Table 1 - The entomofauna distribution across sites in royal salt mines

Insects identified	Wet season	Dry season	Total
Reticulitermes flavipes	8350	5800	14150
Zonocerus elegans	15	5	20
Acraea acrita	2	4	6
Crematogaster sp	109	32	143
Total	**8476**	**5843**	

Table 2 - Heavy Metal Composition Identified in Termites (*Reticulitermes flavipes*)

Heavy metal identified	SITE A	SITE B	Control Site
Zn	0.255 ± 0.280	0.250 ± 0.278	1.74 ± 1.72
PO_4	319.70 ± 329.25	319.40 ± 329.15	13.45 ± 13.40
Hg	0.150 ± 0.150	0.150 ± 0.150	0.010 ± 0.011
Mn	ND ± ND	ND ± ND	Trace ± Trace
Pb	-ve ± -ve	-ve ± -ve	Trace ± Trace
Fe	Trace ± Trace	Trace ± Trace	0.25 ± 0.25
Cu	0.200 ± 0.090	0.180 ± 0.080	Trace ± Trace
Cd	0.200 ± 0.215	0.200 ± 0.215	1.40 ± 1.43

Table 3 - Heavy Metal Composition Identified in Ant *(Crematogaster sp)*

Heavy metal identified	SITE A	SITE B	Control Site
Zn	0.050 ± 0.080	0.045 ± 0.070	0.85 ± 0.85
PO_4	82.850 ± 87.200	82.840 ± 87.190	8.30 ± 8.30
Hg	Trace ± 0.050	Trace±0.050	0.08 ± 0.07
Mn	ND ± ND	ND ± ND	Trace ± Trace
Pb	-ve ± -ve	-ve ± -ve	-ve ± -ve
Fe	Trace ± Trace	Trace ± Trace	ND ± ND
Cu	1.850 ± 2.00	1.845 ± 2.00	0.85 ± 0.85
Cd	0.100 ± 0.150	0.90 ±0.140	0.65 ± 0.65

Table 4 - Heavy Metal Composition Identified in Butterfly (*Acraea acrita)*

Heavy metal identified	SITE A	(b)	Control
Zn	0.300 ± 0.250	0.280 ± 0.240	2.65 ± 2.65
PO_4	120.650 ± 120.650	120.635 ± 120.635	5.15 ± 5.10
Hg	0.065 ± 0.065	0.050 ± 0.050	0.09 ± 0.09
Mn	2.350 ± 1.450	2.345 ± 1.345	1.46 ± 1.49
Pb	-ve ± -ve	-ve ± -ve	-ve ±-ve
Fe	Trace ± Trace	Trace ± Trace	ND ± ND
Cu	7.600 ± 6.60	6.50 ± 7.580	3.44 ± 3.45
Cd	0.350 ±0.450	0.450 ± 0.350	1.85 ± 1.85

Table 5 - Heavy Metal Composition Identified in Grasshopper (*Zonoserus elegans)*

Heavy metal identified	Site A	Site B	Control
Zn	0.160 ±0.140	0.165 ±0.145	1.15 ± 1.15
PO_4	683.70±677.50	683.75 ±677.55	28.2 ± 28.5
Hg	Trace± Trace	Trace ± Trace	Trace ± Trace
Mn	11.00± 10.90	11.00 ± 10.88	5.35 ± 5.35
Pb	0.080±0.080	0.080± 0.075	0.010 ± 0.10
Fe	0.12± 0.10	0.12 ±0.10	0.28 ± 0.28
Cu	1.400± 1.900	1.390 ± 1.890	0.48 ± 0.48
Cd	0.100±0.160	0.170 ± 0.160	1.58 ± 1.55

5 DISCUSSION

Insects are used to study water quality, environmental contaminations, and bioassessment since they are extremely sensitive to environmental changes and capable of disrupting their core functions and activity and changing their life cycle such as growth, metabolism, reproduction, and feeding. Recent studies revealed that insects have been used as bioindicators to monitor ecosystem healthy conditions and plant organisms. Data showed that all four (4) insect taxa clearly showed remarkable species based on variation in metal concentrations indicating metal accumulation patterns for Cd, Ni, Zn, Cu, Fe, Cr, and Mn (Table 2). The highest metal concentrations were observed in *Zonocerus elegans* followed by termites (*Reticulitermes flavipes)* at Site Upper Inyia (SA) respectively. Significantly, site-based differences in the levels of contaminations were observed in the mean concentration of heavy metals at each site; however, it varied up to fivefold between examined species (table 3). However, the lowest metal concentration was seen in *Cremtogaster spp.* recorded at the control site and as well the contaminated site upper Inyia (SA), and lower Inyia (SB) even when it has the closest contact with the contaminated and lead-polluted soil compared to other insects from the control site. This could be a result of its genetic make-up and adaptability to hard substance accumulation. This agrees with Eeva *et al.* (2004) who reported that ants and termites tend to be resistant to severe pollutions. It might also be assumed that ants particularly possess physiological mechanisms, which could aid in the regulation of metal ion concentrations that can prevent metal contamination (Tillberg *et al.,* 2006). However, it should be noted that these metals are persistent and cannot be degraded by insect metabolism, as they accumulate at upper trophic levels.

Furthermore, all four insect taxa clearly showed site and species-dependent metal accumulation patterns for Cu, Pb, Mn, Zn, Cd, Fe, Po_4, and Hg as agreed by Iqra *et al.,* (2015). A high concentration of phosphate was detected on grasshoppers and termites at the contaminated sites (A and B). The higher concentration of phosphate in the insects could be attributed to the mine contamination. This is in agreement with Khan *et al., (*1994) who reported that phosphate composition largely depends on type and parent origin along with environmental polluting elements such as Cr, Cd, As, Sb, V, Zn, Ni, and Cu. This further reveals the negative impacts of mining on insect fauna and human health. It is now obvious that the complaints of ill health by inhabitants of the area (while interacting with the people of the area during the sampling) could be a result of high metal concentration in their body systems. The study further showed the overall diversity and abundance of different study sites. The study results agree with Porrini *et al.,* (2003) who reported insects are used for bioassessment such as monitoring of pesticides, heavy metals, and radionuclides levels. However, the wet season recorded higher abundance compared to the dry season, this agrees with Honnay, (2002) who reported that as average temperature rises, the habitable range for many species is predicted to shift (Honnay, 2002).

8 CONCLUSION

The current study highlighted the degree of threat and danger that mining poses to insects and human lives around the royal Salt mining site, Ikwo, because of increasing metal concentrations of Fe, Mn, Cu, Hg, Pb, Cd, Zn, Po4. The study presents an insight into the scope of different insects that can be used as tools for biomonitoring or bioassessment as well as for studying environmental quality and conditions. This thus advocates a need for proper measures to be taken to reduce the increasing level of environmental pollution (air, water, and soil) by strictly implementing environmental pollution control laws and enforcing proper regulation of mining waste/discharge and other industrial effluents within the mining vicinity.

People of the area should desist from eating termites and grasshoppers around the mining site due to the high level of heavy metal concentration detected as these insects constitute the major local diet of people of that area. There should be community-based awareness to educate and sensitize the villagers on the health implications of the mining activities in the area. Mining poses a serious threat to humans and animals, therefore government at all levels should strive harder to mitigate the effects of the mining through routine monitoring of the activities of the miners thereby ensuring enforcement of all environmental laws in the area.

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