COMPARISON ON BIOACCUMULATION OF HEAVY METALS IN BIRDS AND PLANT SPECIES IN AMURUM FOREST RESERVE AND THE NIGERIAN NATIONAL PETROLEUM CORPORATION (NNPC) REFINERY AREA OF KADUNA, NIGERIA

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Abstract
The bioaccumulation of five heavy metals in birds and plant species was compared between Amurum Forest Reserve and the Nigerian National Petroleum Corporation refinery area Kaduna, Nigeria. Forty-eight tail feathers of birds spread across 16 species belonging to 10 families as well as 8 plants leaves belonging to 3 families were sampled between April and June, 2010. The quantitative measurements of heavy metals: Aluminium(Al), Cadmium (Cd), Lead (Pb), Uranium (U) and Zinc (Zn) were carried out with Inductively-Coupled Plasma Optical Emission Spectrometer (ICP OES). The level of metals concentration in bird feathers as well as plant leaves follow the same trend: Zn > U > Al > Pb > Cd. The concentration of Lead was significantly higher in NNPC. However, there was no significant difference for Al, Cd, U and Zn concentrations between the two study sites. Feeding guilds also showed no significance in each of the study sites like wise age groups and sexes. Metals concentrations in plants leaves showed no significant difference between the two study sites. However, on the average heavy metals concentrations in leaves were higher in the NNPC area except for Cadmium which had a higher concentration in Amurum Forest Reserve. The concentration of metals in feathers of birds were far above the World Health Organization (WHO) recommended Maximum Permissible Limit (MPL) underscoring the need for intensive biomonitoring in highly polluted areas for species conservation management. The same trend was also recorded for the plants with the exception of lead which concentration was below the MPL. The Management of the refinery needs to monitor on a long term, the extent of metal pollution associated with this wasteful practice so as to embark on remediation of the environment.

Keywords: *Heavy metals, birds feathers, plants leaves, maximum permissible limit*

Background to the Study

Many metals are essential for life, in the right concentrations but beyond permissible limit are poisonous (Rainbow and White 1989, Sanders 1997, UNEP 2003). Human population growth is mobilizing heavy metals to the environment in amount similar to, or even exceeding, those introduced through weathering processes (Bruland et al. 1974, Fan 1996, ICES ACME Report 2001), thereby polluting the environment (Depledge et al. 1993). Heavy metals can damage or threaten ecosystem stability at low concentrations and tend to accumulate in the food chain (Albergoni and Piccinni 1983, Battaglia et al. 2005, Ali et al. 2010). Heavy metals pollution is one of the five major types of toxic pollutants commonly present, which accumulate and become persistent in organisms because of their chemical stability or poor biodegradability. Toxic heavy metals are responsible for certain diseases which have lethal effects on both birds and humans. This include lead (Pb), Cadmium (Cd), Copper (Cu) and Zinc (Zn) which have direct effects on the central nervous system, and can cause acute renal failure (Ward et al. 1994). They are readily soluble and environmentally mobile (Mason 1991). Essential heavy metals are generally less toxic than non-essential metals (Batley 1983). The action exerted by non-essential metals is based on chemical similarity to essential metals. For example Cadmium with Copper or Zinc (George 1982) toxicity effects are usually additive and / or synergistic (Depledge 1987). The industry, road traffic, and consumption of fossil fuels are the main source of the global emission and distribution of non-essential heavy metals (Pacyna and Pacyna 2002, Kenntner et al. 2003, Anongo et al. 2005, Bako et al. 2005). The indiscriminate discharge of non-essential heavy metals into the environment ensures their transfer into plants which could result in death of crops or nutrient uptake interference or objectionable deposits on fruits, and renders the edible portion of plants toxic to humans and animals (Bichi 1999). Most metals are global contaminants that biomagnify in water, air and food chains (Lagadic et al.1998) and hence stored in egg shells (Mora 2003), feathers (Veerle et al. 2004), internal tissues such as the liver and the kidney of birds (Braune and Gaskin1987) via blood proteins. For example cadmium binds to low molecular weight of sulfydryl-rich, protein metallothionein and thus accumulates over time (Wenzel et al. 1996, Ek et al. 2004). Persistent uptake of food contaminated by these heavy metals stresses birds. Birds are top predators that often carry high burdens (Perez-Lopez et al. 2008) in food chains, and therefore can provide information over a large area around each sampling site on how, where and when they are transferred within food chain (Jager et al.1996).

Heavy metals such as lead and cadmium are sequestered in the sulfhydryl group of the keratin as feathers grow. The blood supplies atrophies and the metal content in the feather remains extremely resistant to further change once it is fully grown (Burger 1993). The heavy metals in resident birds are derived from food items utilized within home range and these clearly identify the local pollution in the area (Burger 1993, Furness 1993). Plants being stationary in growth habit are usually the first to feel the impact of an altered environment. They can be used in bio-indication. This has been widely applied and validated as a cost effective alternative in air quality assessment and monitoring (Pyatt et al. 1999, IAEA-NAHRES-45 1999, IAEA 2000). Heavy metals are deposited on plant surfaces and on the soil which are gradually taken up through the root and/or stomata (Bako 2000) and subsequently lead to either chronic or acute toxicity symptoms (Taylor 1973). The highest concentration are found in leaves whereas lowest typically observed in seeds with the exception of roots (Ivanova et al. 2003).

The NNPC complex has been refining crude petroleum since 1979 and most of the gas derived from this process has been, and is still being flared (Bako and Ijachi 2007). The refinery has only waste water treatment as part of their plans for the environment for the past three decades, but Bako and Ijachi (2007) have observed the area to be polluted. Recently, human dwellings around the refinery are on the increase, and such residents have little or no idea on the effects of atmospheric gas pollution on their health. Thus, this research was aimed at comparing bioaccumulation of heavy metals by birds and plants in Amurum Forest Reserve, Plateau State and the Nigerian National Petroleum Corporation (NNPC) refinery area, Kaduna State, Nigeria.

Materials and Methods

Study Sites

The study was carried out at Amurum Forest Reserve (AFR) (9°53′N, 8°59′E) in Plateau State (Ezealor 2002) and the NNPC refinery (10°24′N, 7°29′E) located in the southern part of Kaduna town (Bako and Ijachi 2007).

Duration of study

This study was carried out between April-June 2010. Four weeks was spent at each study site.

Sampling of Birds

To collect feathers samples, nine 12 m mist-nets were used to trap birds in the study sites. The mist-nets were opened and closed in the mornings between 5:30am to 9:30am. All birds trapped were identified, grouped according to their feeding guilds (Karunagaran and Subramanian 1999), aged and sexed. For each trapped bird the second outer tail feather from the left was collected and placed in an envelope. The envelopes were labeled and stored under room temperature between the range of 23°C and 25°C for analysis. A total of 48 birds' feathers were collected from both sites.

Sampling of Plant Material

The plant species selected for this study include Dichrostachys cinerea (Syn: D. glomerata: Leguminosae: Mimosoideae), Parkia biglobosa (Leguminosae: Mimosoideae), Ficus platyphylla (Syn: F. umbrosa: Moraceae) and Mangifera indica (Anacardiaceae), which are widespread in tropical Africa and common in savanna regions, where they grow as large shrubs and are sources of food, fodder and medicine to the indigenous communities and their livestock (Bako and Ijachi 2007). A twig was collected about 1-2 meters from the base of the crown of each of four tree species. Ten fully expanded leaves were removed from basal part of each twig and the samples were bulked for each tree in each site. Samples were sealed in clean polythene bags, labeled and taken to the laboratory.

Sample Preparation and Chemical Analyses

Feathers were vigorously rinsed twice with distilled water alternated with 1mol/L acetone to remove external contamination (Gochfeld et al. 1996).The samples were put in envelopes, and dried in oven at 60°C for 24 hrs. The dry feathers were put in crucibles and weighed using a digital scale balance (Adventurer Ohaus, Item AR2140, Max Cap: 210g N13123, C203797US, Readability: 0.0001g, SN: 1227031206, Ohaus Corp. Pine Brook, NJ USA, made in China) to the nearest 0.0001mg. Subsequently, a mixture of 2ml HNO3 and 6ml HCl was added to the dried samples to begin digestion. The samples were heated at 250°C using a sand bath inside the fume cupboard for 6 hours and the feathers were allowed to digest. After digestion, samples were removed and diluted with 2 ml HCl to dissolve the digested samples and 20 ml of distilled water was added and then filtered using a funnel and filter paper inside 100 ml volumetric flask with distilled water.

The leaf samples were washed carefully in running tap water and rinsed twice with distilled water. These were oven dried at 60°C for 48 hours in labeled, large paper envelopes (Bako and Ijachi 2007). Dried samples were ground to fine powder in agate mortar. For each sample, 1g of ground fine powder was placed in a crucible. 10 ml of concentrated HNO3 was added and allowed to stand overnight (for 24hrs). The samples were heated at 250°C until the production of red Nitrogen dioxide (NO2) fumes ceased. The samples were allowed to cool and 2 ml of 70% HClO4 (Perchloric acid) was added and heated again, and allowed to evaporate to small volume. The samples were filtered in 100 ml volumetric flask with distilled water. An Inductively Coupled Plasma Optical Emission Spectrometer (ICP OES) was used to analyze for metals (Hall and Fisher, Jr. 1985, Marc et al. 2009, http://research.uiowa.edu/nniui/ICPinfo).

Maximum permissible limits of metals in birds and plants

All the metals detected in birds were compared to the maximum permissible limits for metals (Burger 1993).The WHO maximum permissible limit (MPL) standard for plants as cited in Oyewo (2001) were used for comparison with the metals detected in plants. The WHO standards were obtained using the Atomic Absorption Spectrometer machine.

Statistical Analyses

The R-console software was used for statistical analyses. Independent sample T-test was used to compare concentration of heavy metals in plants and birds between study sites and also, between sexes and age groups in birds. One-way Analysis of Variance (ANOVA) was used to compare concentration of heavy metals between feeding guilds. Significant level was achieved if $P < 0.05$.

Results

A total of 48 birds' feathers spread across 16 species belonging to10 families as well as 8 plants leaves belonging to 3 families were analyzed for metals across study sites. Sixteen of the 48 feathers were from frugivorous birds, 20 from granivorous birds and the remaining 12 were from insectivorous birds. The level of metals concentration in bird feathers as well as plant leaves follow the same trend: Zn > U > Al > Pb > Cd (Table 1 and 2). There was no significant difference in the concentration of heavy metals in feathers between species in the two study sites (Fig. 1): Aluminium (Al) ($t = 0.3243$, $df = 46$, $p = 0.7472$), Cadmium (Cd) ($t = 0.7239$, $df = 33.192$, $p = 0.4742$), Uranium (U) ($t = 0.1942$, $df = 46$, $p = 0.8469$) and Zinc (Zn) ($t = 0.4947$, $df = 46$, $p = 0.6232$), with the exception of Lead (Pb) which showed a significant difference between species in the two study sites ($t = -2.081$, $df = 34.379$, $p = 0.0450$, Fig. 1).

The concentration of metals between the study sites was not significantly different in each feeding guild. However, with the exception of Lead for Amurum Forest Reserve, there was no significant difference in the concentration of all heavy metals tested across feeding guilds within sites. There was no significant difference in metals concentration in feathers of birds between the two age groups and sexes in each study site. There was no significant difference in the concentration of heavy metals in plants leaves across the study sites. Mean concentration of metals in species compared with maximum permissible limits of three metals in birds and plants The concentrations of Cadmium, Lead and Zinc in the sixteen bird species across the study sites were above the maximum permissible limits. Also, in the four plant species the concentrations of Cadmium and Zinc across the study sites were above the maximum permissible for Cadmium and Zinc, but below maximum permissible limit for Lead.

Table 1: Metals level in birds across study sites

Metals	n	Amurum Forest Reserve Mean ± S.E. (ppm)	NNPC Area Mean ± S.E. (ppm)	WHO MPL (ppm)
Zinc	24	124,814.92±48,100.83	99,180.83±19,278.94	10
Aluminium		4,746.40±997.72	4,340.98±753.43	
Uranium		26,307.08±3,631.14	25,306.83±3,654.72	
Lead		136.84±24.77	249.50±48.14	5
Cadmium		136.11±39.06	104.70±18.88	0.1

Table 2: Mean concentration of metals in plants leaves across study sites

Metals	Amurum Forest Reserve Mean ± S.E.	NNPC Area Mean ± S.E.	n	df	t	p	WHO MPL (ppm)
Aluminium	158.25 ± 17.76	162.68 ± 16.41	4	6.00	-0.18	0.86	
Cadmium	0.87 ± 17.76	0.25 ± 16.41		3.00	0.98	0.40	0.5
Lead	1.16 ± 17.76	1.72 ± 16.41		6.00	-1.21	0.27	7
Uranium	349.78 ± 17.76	523.43 ± 16.41		3.17	-1.34	0.27	
Zinc	1,658.50 ± 17.76	2,771.75 ± 16.41		6.00	-1.18	0.28	20

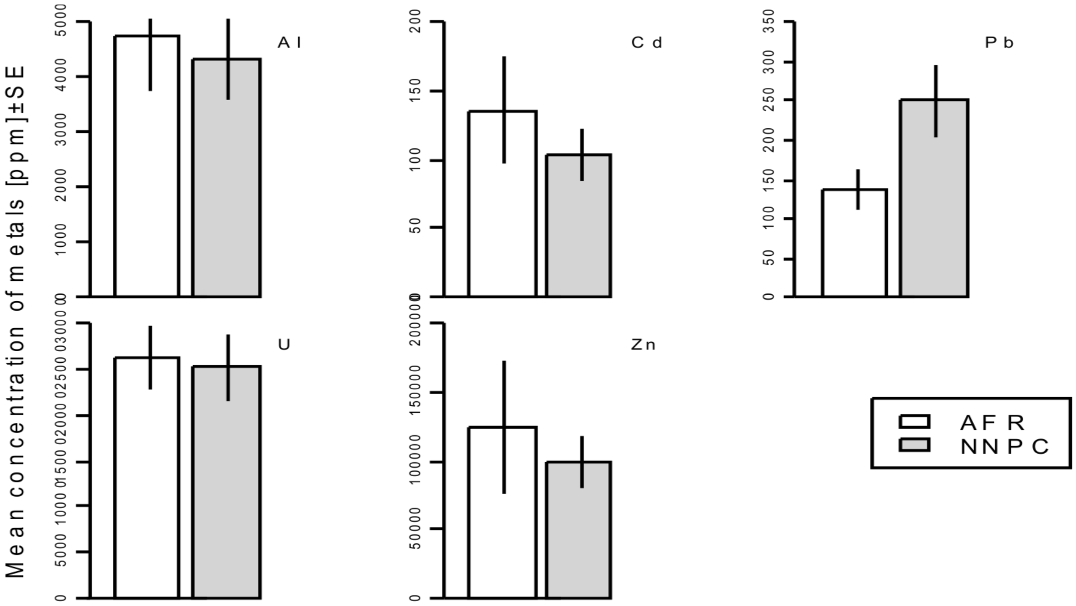

Figure 1: Mean concentration of metals in feathers between the two study sites

Discussion

The level of concentration being highest in Zinc and least in Cadmium (Table 1 and 2) agrees with Janssens et al. (2001) report on heavy metals in feathers of great tits (Parus major). With the exception of Lead, the concentration of Zinc, Uranium, Aluminium and Cadmium did not vary across the two study sites. But, concentrations of these metals on the average were higher in Amurum Forest Reserve than the NNPC area. This do not imply high potential toxicity to the environment due to its geogenic origins in sediments (Xu et al. 2009) compared to the NNPC area subject to atmospheric pollution due to gas flaring from the refinery. This is most likely due to wind-blown mineral dust from quarrying site at Furaka village which is about 8 km away from the reserve. Dauwe et al. (2002) reported that one of the sources of environmental contamination is from dust from ore piles blown up by the wind.

The concentration of Lead in the feathers of birds differs significantly between the two study sites; this probably is due to gas flaring by the NNPC refinery. This is in accordance with O'Meara et al. (1986) and Dauwe et al. (2002) which showed that the concentration of Pb was greater in polluted site (near a metallurgic factory) than at the control site. In this study, the average Lead concentration in the NNPC site was about two times higher than Amurum Forest Reserve. For over a century, high lead exposure in birds has been a concern as cited in studies by Perez-Lopez et al. (2008). In Nigeria, the disposal of natural gas by flaring is widely practiced, with no available data on the extent of metal pollution associated with this wasteful practice. Among heavy metals, Lead emission is considered the most serious effect of pollution (Hutchinson and Meema 1987). Recently, the illegal excavation of soil contaminated with Lead for gold in Zamfara State, Nigeria resulted in Lead poisoning, which had killed over 355 people than the whole world combined records in the last 40 years (http://www.france24.com/en/20100611-...ic-hit-nigeria). Thus, the high concentrations of Lead observed in feathers as shown in this study might be a good index of the health of the two environments studied and may have lethal implications as Lead concentrations in feathers (specifically tail feathers) has been shown to correlate significantly with concentrations in internal tissues and other feathers considered in a study on the great and blue tits feathers as biomonitors for heavy metal pollution by Dauwe et al. (2002). In the long term, through the effect of bioaccumulation (Perez-Lopez et al. 2008), this might have more severe consequences in non-migratory species that carry out their entire life functions within a confined area which include bronze mannikin and village weaver as recorded in the study.

The granivorous birds in the NNPC area had the highest concentration of metals which could be due to absorption of flared gases (atmospheric deposition) by cultivated plants (cereals) and grasses. Janiga (2008) reported that granivorous birds have a higher degree of exposure to metal contaminations, especially, Lead in the environment. The frugivorous birds in Amurum Forest Reserve had highest concentration of all metals. This is probably due to high absorption rate by the fruit producing plants in the reserve as well as wind-blown dust from adjoining quarry that settles on fruits that are ingested by frugivorous birds. The similarity in heavy metal concentrations between age groups suggests that there is no age-related difference and that external contamination may be an important source for heavy metals in feathers (Dauwe et al. 2002).

The lack of variation in the concentration of metals between sexes clearly suggests that any bird, irrespective of the sex, could play the role of bioindicator (Devkota and Schmidt 2000). Scanlon et al. (1980) and Dauwe et al. (2002) also reported that mean concentrations did not vary significantly between sexes. The concentration of metals in plant leaves across study sites showed no variation. However, on the average the concentration of metals was higher in NNPC except for Cadmium. This probably is due to atmospheric deposits from the NNPC refinery being utilized by the plants. While Amurum Forest Reserve had higher concentration for Cadmium, possibly due to forest fires (Bull 2010) and weathering of rocks (Parmeggiani 1983). All the birds analyzed in this study have heavy metal concentrations that are above the maximum permissible limits for adverse effects on birds species. With the exception of Lead, the plants species analyzed have heavy metal concentrations that are above the maximum permissible limits of Cadmium and Zinc.

Conclusion

In this study, there was distinct difference between the two study sites in the concentration of Lead in feathers of birds. However, diet has no significant effect on heavy metals concentration in birds species. Also, there was no gender- or age-related difference in the metal levels in the tail feathers of birds, suggesting that external contamination onto the feather surface may be an important route of heavy metals in the feather. The data on concentration of metals in leaves of plants suggest that leaves may be very useful biomonitors for local contamination with heavy metals. All birds analyzed were above the maximum permissible limit which is an indication that the two study sites are contaminated. The Management of the refinery needs to monitor on a long term, the extent of metal pollution associated with this wasteful practice so as to embark on remediation of the environment. Also, they should advise residents around the refinery area to know the danger of gas flaring on their health and the need to relocate from the area.

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