ECOLOGICAL RISK ASSESSMENT OF HEAVY METALS IN INTEGRATED POULTRY-FISH FARMING

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ABSTRACT
This report gives a preliminary assessment of ecological risk presented by heavy metals; cadmium, chromium, copper, lead and zinc in integrated poultry-fish farming. Water and sediment samples taken from a fish pond receiving poultry (chicken) manure as part of the feed were analysed for physicochemical properties and levels of the heavy metals. The heavy metals content of the sediment samples were found be markedly higher (M_{Total} 10.70±1.60 mg.kg^{-1} and contained 0.80±0.10 mg.kg^{-1} As; 1.00±0.30 mg.kg^{-1} Cd; 0.60±0.10 mg.kg^{-1} Cr; 2.40±0.10 mg.kg^{-1} Cu; 1.00±0.20 mg.kg^{-1} Pb and 4.90±0.90 mg.kg^{-1} Zn) than in the pond water (M_{Total} 0.85 mg.l^{-1}) giving values of partition coefficient, measured in terms of the ratio of the levels of metals in the pond to the levels in the sediment of 0.08 with respect to the total levels of the metals and as low as 0.04 for Cu and Zn. The levels of the heavy metlas (M_{Total} 2.80±0.21) in the poultry manure were higher than in the pond water but markedly lower than in the sediment (M_{Total} 10.70±1.30 mg.kg^{-1}). Five fish were taken from the pond, sacrificed; the fills and flesh taken from the fish trunk were dried. The levels of the heavy metals in the fish samples were generally low; M_{Total} 5.462±0.792 mg.kg^{-1} containing 0.758±0.008 mg.kg^{-1}dw As; 2.880±0.648 mg.kg^{-1}dw Zn; 0.126±0.001 mg.kg^{-1}dw Cr; 1.240±0.098 mg.kg^{-1}dw Cu; 0.322±0.018 mg.kg^{-1}dw Cd and 0.136±0.020 mg.kg^{-1}dw Pb, with the levels of the heavy metals in the fish gills expectedly higher than in the fish tissues. The levels of the heavy metals in the fish tissues were generally higher than in the pond water giving values of bioaccumulation index greater than 1.0 but less than 2.0. The levels of the heavy metals in the fish tissues were found to be lower than their toxicity reference values giving values of hazard quotient lower than 1.0 and suggesting that the heavy metals may not present imminent ecological concern.

INTRODUCTION
Fish is a major source of dietary protein for man. A recent report indicated that in developing countries, fish may be an important component of the solution to the prevalent dietary protein shortage (Nnaji *et al*., 2009). The need for low-cost fish production system is provided for by integrating fish farming with other agricultural production, such as livestock. Integrated fish farming systems may be categorized into: integrated animal-fish farming (and this includes integrated poultry (chicken-fish farming); integrated animal-plant-fish farming; integrated plant-fish farming and integrated wastewater-fish farming (Nnaji *et al*., 2003). The direct utilization of livestock (cattle, pig, and chicken) manure as feed for fish has been widely studied (Fowler and Lock, 1974; Lu and Kevern, 1975; Oladosu *et al*., 1990; Little and Edwards, 2003, Nnaji *et al.,* 2009). Fresh poultry manure contains about 75-80% water, but as a manure of the overall dry matter, 5% is N, 3% is P and 2.4% is K (Kroodsman, 1986). Approximately 60-70% of the total nitrogen in poultry manure occurs as ceric acid and urea (Nahm, 2003). Chicken manure, when added into a pond undergoes microbial decomposition releasing nutrients for the growth of phytoplankton which is the base of the food chain (trophic level) in aquatic systems. Phytoplankton is eaten by zooplankton which serves as food for small fish and aquatic insects. Thus the addition of poultry manure stimulates the production of primary productivity (phytoplankton and zooplankton) in the pond. The primary nutrients released by microbial decomposition of poultry manure are N, P, and K; with N and P being the nutrients most likely to limit plankton growth in the pond and fish yield will more probably be directly correlated to manure nitrogen content since N is more liable/volatile than phosphorus. Secondary nutrients in poultry manure include Ca, Mg and S, while heavy metals constitute minor nutrients (Boyd, 1982; Nnaji *et al*., 2011). Integrated livestock-fish farming may be associated with ecological and human

health concerns of potential transfer of (i) pathogens from livestock to fish (which can reduce fish growth rate and resistance to disease, and cause fish mortality) and to humans and (ii) heavy metals in livestock manure to man.

Heavy metals in livestock manure are derived mainly from the animal feed. A number of metals are added to poultry feedstuff not only as essential nutrients but also as supplement to improve heath and feed efficiency. Essential nutrient elements participate in a wide range of enzymatic processes involving many aspects of physiological functions and intermediary metabolism of the organism (Bolan *et al.*, 2004). They act as catalysts of co-factors in enzyme systems with their roles ranging from relative weak, non-specific ion effects (metal ion activated enzymes) to high specific associations (metallo-enzymes) in which the metal id firmly attached to the protein in a fixed number of atoms per molecule. Improving feed efficiency and health of animals is important consideration in intensive, confined animal production system. Because of the intensity of production, a number of feed additives are used to reduce outbreaks of diseases (Sims and Wolf, 1994; Moore *et al.*, 1995). Among the many feed additives, the metals As, Co, Cu, Fe, Mn, Se and Zn are added to prevent diseases, improve weight gains and feed conversion, and increase egg production (Miller *et al.*, 1995, Tufft and Nockels, 1991). A major proportion of the ingested metals is excreted in urine and faeces and gets incorporated in the manure by-product (Cang *et al.*, 2004). Although integrated poultry-fish farming is practiced in Nigeria, there are no reports, to the best of our knowledge, of studies to evaluate the associated potential ecological risk. The objective of this study was to provide exploratory data and information on the risk potential of heavy metals in integrated poultry-fish farming.

MATERIALS AND METHODS

Poultry manure that is used as part of the feed in a fish pond in Ughelli, Delta State was collected and analysed for heavy metals using standard procedure. In a typical experiment a known amount of the dry poultry manure was digested with 4M HNO_3. The mixture was cooled and filtered through a Whatman filter paper and the filtrate was analysed for As, Cd, Cr, Cu, Pb and Zn using AAS (Buck Scientific VGF model 201A). The mineral nutrients content of the poultry manure were determined by chemical methods (AOAC, 1990). The mineral nutrients content together with the levels of the heavy metals in the poultry manure are given in Table 1.

Table 1: Mineral nutrient content and levels of heavy metals in poultry manure

Parameters	
Ca^{2+} (%)	8.60±1.00
Mg^{2+} (%)	1.70±0.30
Na^{+} (%)	0.70±0.10
K^{+} (%)	0.38±0.10
P ($mg.kg^{-1}$ dw)	7.30±1.10
N (%)	3.40±0.30
As ($mg.kg^{-1}$ dw)	0.40±0.01
Cd ($mg.kg^{-1}$ dw)	0.20±0.01
Cr ($mg.kg^{-1}$ dw)	0.10±0.01
Cu ($mg.kg^{-1}$ dw)	1.201±0.20
Pb ($mg.kg^{-1}$ dw)	0.10±0.01
Zn ($mg.kg^{-1}$ dw)	1.80±0.20

Water and sediment samples were collected from the fish pond in quadruplates. The sediment samples were air-dried, ground and sieved through a 20µm screen. Equal amounts of the water and sieved sediment samples were pooled into aggregate samples. The physicochemical properties of the pond water were determined by using the standard procedures (APHA, 1992) while the heavy metals content of the pond water and sediment samples were determined using AAS.

Five fishes (*Clarias gariepinus*) were removed from the fish pond with a net and sacrificed. The gills and flesh from the fish trunk were removed and dried at 60°C and thereafter ashed in a Muffle furnace at 500°C form 3h. The resulting ash was digested with dilute HCl, the digestate was filtered and the filtrate analysed for the heavy metals using AAS.

RESULTS AND DISCUSSION

Mineral content and levels of heavy metals in poultry manure

The composition; mineral nutrient content and levels of heavy metals in poultry manure, largely depends on the feed composition, efficiency of feed conversion and on the intensity of health care measures for the poultry. The results in Table 1 show that the levels of the primary nutrients, N, P and K in the poultry manure are relatively high; 3.40±0.30 N; 7.30±1.10 mg.kg^{-1}dw P and 0.38±0.10% K; with the values of the secondary nutrients; Ca^{2+} 8.60±1.00% and Mg^{2+} 1.70±0.30% being relatively higher. The levels of the heavy metals in the poultry manure varied from 0.10±0.01 mg.kg^{-1}dw for Pb^{2+} and Cr^{3+} to 1.80±0.20 mg.kg^{-1}dw for Zn. The levels of heavy metals in the poultry manure used in this study are higher than the values reported by Cang *et al*. (2004) for poultry livestock feed and manure in the Jiangsu area of China, but are markedly lower than the values reported in a more recent study (Okieimen and Wuana, 2009) for poultry manure in the Makurdi area of Benue State which revealed that a large proportion of the heavy metals in the manure were in the water soluble and exchangeable forms in which they could present significant ecological/environmental concern. In a comprehensive review of heavy metals in poultry manure, Sims and Wolf (1994) summarized findings from previous research projects: Zn concentrations ranged from non-detectable (nd) to 660 mg.kg^{-1} dm in poultry manure to and from nd to 699 mg.kg^{-1}dm in poultry litter. Cu concentrations in poultry manures were in the range nd – 232 mg.kg^{-1}dm, the high Cu concentrations in some of the manures being ascribed to copper sulphate supplements added to the diet. Fleming and Mordenti (1991) reported average Cu concentrations of 59 mg.kg^{-1} dm in Belgian poultry manures while median Zn and Cu concentrations in Swiss poultry manures were 349-511 mg.kg^{-1} dm and 35 – 44 mg.kg^{-1}dm respectively (Menzi and Kessler, 1998).

Physicochemical properties and heavy metal content of pond water

The physicochemical properties and heavy metals content of the fish pond water are given in Table 2. A careful examination of the results in Table 2 shows that the total dissolved solids (TDS) value of the fish pond water (480.00±10.00 mg.l^{-1}) is markedly high in comparison with the value of conductivity (0.01±0.00μs.cm^{-1}) of the pond water; may be indicative of the nature of the solids content of the pond water, that is, the solids content of the fish pond water may not be ionic or ionizable substances.

Table 2: Physico-chemical properties and heavy metals content of fish pond water

Parameters	
pH	6.60±0.01
Conductivity (μs.cm^{-1})	0.01±0.00
Turbidity (NTU)	4.00±0.01
Total dissolved solids (mg.l^{-1})	480.00±10.00
Dissolved oxygen (mg.l^{-1})	11.40±1.20
Chemical oxygen demand (mg.l^{-1})	56.60±4.90
Biochemical oxygen demand	2.60±0.40
Ca^{2+} (mg.l^{-1})	34.10±1.20
Mg^{2+} (mg.l^{-1})	4.90±1.00
Na^{+} (mg.l^{-1})	0.30±0.01
K^{+} (mg.l^{-1})	1.40±0.30
NO_3^- (mg.l^{-1})	4.40±0.80
SO_4^{2-} (mg.l^{-1})	0.80±0.10
PO_4^{3-} (mg.l^{-1})	3.40±0.20
As (mg.l^{-1})	0.30±0.05
Cd (mg.l^{-1})	0.01±0.00 (0.02)
Cr (mg.l^{-1})	0.04±0.00 (0.05)
Cu (mg.l^{-1})	0.10±0.02 (1.00)
Pb (mg.l^{-1})	0.20±0.00 (0.10)
Zn (mg.l^{-1})	0.20±0.02 (15)

Values in parentheses are the WHO maximum allowable limit (mg.l^{-1})

Conductivity of water, a more-or-less linear function of the concentration of dissolved ions, is not of itself a human or aquatic health concern but because of its ease of measurement, it provides an indication of other water quality issues. The pH of water affects the solubility of many toxic and nutritive chemicals' therefore the availability of these substances to aquatic organisms is affected. As acidity increases, many metals become more water soluble and more toxic. The desirable pH range for fish water is 6.5 – 9.5 while the acceptance range is 5.5 – 10.0 (Stone and Thomforde, 2003; Ehiagbonare and Ogunrinde, 2010). The pH obtained for the pond water under study (6.60±0.01) lies within the acceptable range and compares with the pH range of 6.9-7.1 reported for fish pond water in Okada and environs (Ehiagbonare and Ogunrinde, 2010) and 56.60±4.90 mg.l^{-1} respectively, generally are indicative of "grey" water quality and are as would be expected. Dissolved oxygen, a measure of the amount of gaseous oxygen dissolved in an aqueous medium plays a vital role in the biology of cultured organisms (Dhawan and Karu, 2002). The statutory DO level adequate for sustaining aquatic life is 5 $mg.l^{-1}$ while DO values lower than 2 $mg.l^{-1}$ may lead to death of many fish (Omole and Longe, 2008). The measured DO value of 11.40±1.20 for the fish pond water indicates that the water suggests that the water is sufficiently good quality in respect of oxygen requirement for culturing fish. The levels of the heavy metals in the pond water are generally low and below the WHO maximum allowable limit except for Pb^{2+} for which the measured value is 50% higher than the allowable maximum level. These results indicate that Pb^{2+} with a hazard quotient of 2.0 may be the only heavy metal with possibility of ecological concern in the pond water; while Zn^{2+} with a hazard quotient of 0.013 is the metal with the possibility of ecological risk in the pond water.

Levels of heavy metals in fish pond sediment

Table 3 gives the levels of the heavy metals in fish pond sediment. Sediment quality plays an important role in intensive fish farming as the concentration of a substance (contaminant) may build up in the sediment and then act as source of the contaminant even the primary source of the discharge is removed. Where the substance has low water solubility and a high bioconcentration potential (measured by octanol/water partition coefficient) flow of the contaminant from the sediment through the water to organism may occur without the substance reaching an analytically detectable level in water. The results in Table 3 indicate that the levels of the heavy metals in the pond sediment are markedly higher than the corresponding values in pond water, giving values of distribution coefficient, measured in terms of the ratio of metal content in pond water to the corresponding level in pond water, generally lower than 1.0; which ranged from 0.01 for cadmium to 0.37 for As.

Table 3: Levels of heavy metals in fish pond sediment

Heavy metals	Levels in sediment ($mg.kg^{-1}$ dw)
Arsenic	0.80±0.01
Cadmium	1.00±0.03
Copper	2.40±0.10
Chromium	0.60±0.10
Lead	1.00±0.20
Zinc	4.90±0.90

These relatively high levels of the metals in the pond bottom sediment may have direct impact on the health of the aquatic (fish pond) ecosystem the fish, *Clarias gariepinus*, under culture is bottom-dwelling, bottom-feeding. Sediment is the major repository of metals, in some cases, holding more than 99% of total amount in aquatic system (Odiete, 1999). The total metal burden of the fish pond sediment (M_{total}) of 10.70±1.30$mg.kg^{-1}$ is relatively high and if it is mainly due to the application of poultry manure as feed supplement represents significant fish pond sediment quality impairment. It is pertinent to observe that the relative prevalence of Zn in the pond bottom sediment (4.90±0.90 $mg.kg^{-1}$dw) may be reflective of the level of the metal in the poultry manure (1.80±0.20$mg.kg^{-1}$ dw) used to supplement the feed for the fish.

Levels of heavy metals in fish tissues

The levels of the metals in the fish are given in Table 4. Fish may accumulate heavy metals to various extents depending on species, age, season, feeding habit and the concentration of the heavy metal in the pond. Fish may absorb dissolved mineral elements and trace metals from its feeding and surrounding water, through their body surface, the highly permeable gill epithelial membranes and through ingestion/ gastrointerstinal absorption (McCarthy and Shagart, 1990); with the gills tending to accumulate more heavy metals than the flesh.

Table 4: Average concentration levels of heavy metals in fish tissues ($mg.kg^{-1}$ dw) n = 5

Heavy metal	Levels of heavy metals ($mg.kg^{-1}$ dw)		
	Gills	Flesh	Total
As	0.450±0.048	0.308±0.048	0.758±0.008
Cd	0.216±0.014	0.106±0.004	0.322±0.018
Cr	0.086±0.002	0.040±0.002	0.126±0.001
Cu	0.900±0.048	0.340±0.050	1.240±0.098
Pb	0.120±0.018	0.016±0.002	0.136±0.020
Zn	1.720±0.340	1.160±0.308	2.880±0.648
total	3.492±0.470	1.970±0.414	5.462±0.773

The results in Table 4 show that the total metal (M_{Total}) in the fish is 5.46±0.773 $mg.kg^{-1}$ with the mean values of As, Cd, Cr, Cu and Pb in the gills significantly different ($P < 0.05$) from the levels in the flesh. The level of Zn in the gills was found not to be significantly different ($p < 0.05$) from the level in the flesh. The apparent trend in the level of the heavy metals in the fish in Zn > Cu > As > Cd > Pb > Cr. The relative prevalence of As, Cu and Zn in the fish tissues may be related to their being more likely to be components of fish feed and/or poultry healthcare products. The levels of these metals (As, Cu and Zn) in the fish tissues appear well correlated with the amount in poultry manure (Table 1).

The potential for a contaminant to be an ecological risk may be determined by its bioaccumulated quotient (BQ) given by the ratio of the contaminant in parts or in whole organism to the level in water, the bioaccumulation of As, Cd, Cr, Cu, Pb and Zn in the whole fish is given in Table 5. The value of BQ may not be adequate/sufficient to provide insight into potential ecological risk of a contaminant. For example, the BQ value may be overestimated in the case of a contaminant with limited water solubility that is ingested, digested and absorbed from the sediment by bottom-feeding organism. Accumulation of the contaminant in this case is not mediated through the water body but due to the feeding habit of the organism.

The results in Table indicate that the levels of the heavy metals in the fish were generally higher than in the pond water, giving values of bioaccumulation quotient ranging from 0.68 for Pb to 32.20 for Cd.

Table 5: Ecological risk indices of heavy metals in fish in poultry-fish pond

Heavy metal	Ecological risk index		
	BQ	MAR	HQ
As	2.53	-	-
Cd	32.20	0.062	0.16
Cr	3.15	21.00	0.84
Cu	12.40	0.24	0.41
Pb	0.68	2.94	0.07
Zn	14.40	5.36	0.04

BQ = bioaccumulation quotient; MAR = maximum acceptable risk level, HQ = hazard quotient

The maximum acceptable risk level (MAR) is an ecological risk index that provides a simplified representation of biomagnification in food web. It is given by the ratio of the dietary no observed effect concentration in man (maximum allowable limit) to the bioaccumulation quotient (Ramjin *et al.*, 1994). Values of MAR > 1 indicate high maximum acceptable risk level while values of MAR < 1 indicate low maximum acceptable risk level. The MAR values of As, Cd, Cr, Cu, Pb and Zn in the fish tissues are given in Table 5. The results show that the metals with the potential to biomagnify in man assuming that the investigated fish is consumed are Cr, Pb and Zn with MAR values greater than 1.0.

Hazard quotient (HQ) is an ecological risk index that expresses the potential of a contaminant being an ecological risk or a contaminant of potential ecological concern. The HQ is given by the ratio of the measured concentration of the contaminant to the toxicity reference value or selected screening benchmark (Brooks *et al.,* 2003; LeCoultre, 2001). The values of HQ obtained for the heavy metals in this study are given in Table 5. The results show that the HQ values are lower than 1.0 and indicate that the heavy metal burden of the fish in pond receiving poultry manure as feed supplement is lower than the maximum allowable values, and that the heavy metals may not present imminent ecological concern. However, long-term chronic effects of heavy metals on fish physiology and reproduction together with the potential for transfer to man remain in poultry-fish farming.

CONCLUSION

In this study, the potential for the heavy in the poultry manure used as fish feed in integrated poultry-fish farming to present ecological risk was investigated. The results showed that the fish pond water was of relatively good quality, particularly with respect to the values of oxygen demand indices and levels of As, Cd, Cr, Cu, Pb and Zn, which were well within allowable limits. The relatively higher levels of the heavy metals in the pond sediment and fish tissues than the pond water correlated well with their levels in the poultry manure. Frequent removal of sediment from fish ponds receiving poultry manure as feed is recommended to mitigate/preclude the retention and redistribution of its heavy metals content to other environmental receptors.

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