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Seasonal Speciation Study of Heavy Metals Content in Well Water of Some Chicken Farms in Osun State, Southwestern Nigeria

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Abstract Heavy metals are native to various soils, dusts, air, sediments, water and plant constituent in various means and these relations quantify their mobility and availability. In this manuscript, the seasonal differences in contents of arsenic, cadmium, copper, iron, lead and zinc in well water within the proximity of three main chicken farms in Osun State, were studied to ascertain the ecological impacts of the chicken farming on the well water sources. Water samples taken for eight months were assayed for pH, conductivity, total dissolved solids, alkalinity and speciation of heavy metal contents utilizing standard techniques. A column filled up with Amberlite XAD-16 resin was utilized for the quantification of metals bound to humic substances and free metal ions in the water samples. The samples were assayed for their heavy metal content utilizing Flame Atomic Absorption Spectrophotometer. Quality assurance techniques involved blank determination, recovery analysis and calibration of standards. Descriptive and inferential statistics were used for data interpretations. The content ranges of the metals were: 0.0110–0.0878, 0.0130–0.0900, 0.1124–0.5196, 17.0563-59.4264, 0.021-0.2283 and 3.0420–21.1450 µg/mL for As, Cd, Cu, Fe, Pb and Zn, respectively. With the exception of Cu, the content ranges of all the other metals were present to be exceeded the international standards and acceptable level for drinkable water. The speciation analyzes signified that Fe and Zn were present primarily in the particulate phase, As and Cu were predominant in the humic substances phase, while Cd, Fe, Pb and Zn mainly found in the free metal ions. The free metal ions phase of Cd, Fe, Pb and Zn were found to be bio-available, and thus, could present health risks to humans and poultry bird. The contents found from the control site signified that the chicken farm activities might have impacted the well water with high heavy metal burdens.

Keywords: *seasonal speciation study, chicken farms well water, bioavailability, heavy metals*

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1. Introduction

Confined animal feeding operations impact all main ecological matrices, such as air, soil water and vegetation. Of great importance interest are the pollution of well and open water resources with nutrients, heavy metals, industrial and agricultural chemicals, and microorganisms. Inappropriate management of poultry dropping and other agricultural wastes causes water and air deterioration, which in sequence impact both the water and the land ecosystems encompassing these operations [1]. Heavy metals are intrinsic in various soils, dusts, air, sediments, water and plant constituent in various means and these relations quantify their mobility and availability.

The quantification of total metal content of water and soil samples is not enough to assess the potential mobility and bioavailability of noxious metals to a living organism. Environmental science community identifies that total content is not an exact estimator of the bioavailability or chemical reactivity of a particular pollutant in soils, sediments or aquatic ecosystems. Total metal determinations do not inevitably amount to metal bioavailability. Instead, the toxicity of a material be it an element, an ion or a molecule usually is determined by the physicochemical states of the metal, particularly the measure of free metal ions and very reactive complexes under the different range of forms exist in natural ecosystems [2]. For instance, sulfide minerals may be enclosed in quartz or other chemically inactive rock deposits, and in spite of high total contents of metals in sediment and soil comprising these inorganic substances, metals are not easily available for absorption in the ecosystem [2]. So as to determine effects and possible risks intrinsic in elevated elemental contents that origin from natural disintegration of rock deposits or from poultry activities, the phase of total elemental loads in water, sediment, and soil that are bioavailable must be recognized. Bioavailability is the amount of total metals that are available for integration into ecosystem.

Speciation of heavy metals in water is achieved by the use of ion exchange resin to choosily set apart the exchangeable ions from other phases. More recent resins and methods are continually being devised to enhance analytical sample recoveries quantifiably [3]. Some of these recently devised resins are Amberlite-XAD 2, 8 and 16 which are very good in speciation analysis of dissoluble ions [4]. In accordance with Oyekunle et al. [5] there is shortage of publication on the contents of heavy metal present in Southwestern Nigerian well waters. This has been attributed to somewhat few researches that have been embarked upon in Nigeria addressing contents of heavy metals species in well waters.

The aims of this research were to assess the heavy metal bioavailability and chemical speciation in the analyzed well water, and evaluate the hazards caused by drinkable well water contaminated with heavy metals.

2. Materials and Methods

2.1. Sampling Site

The study field included Ejigbo, Isundunrin and Osogbo chicken farms in Osun State, Southwestern Nigeria. The map of the area under study is displayed in Figure 1 while Table 1 presents the geographical locations of the sampling sites. The study field situates within longitudes 004^0 16.095 ´to 004^0 30.826´E and latitudes 07^0 45.195´and 07^0 53.961´N, whilst the ground level is within 311.81 to 357.23 m above water level. In this manner, the depth of the artesian aquifer stratum differs along with the land topography. The indwellers of the farm count basically on well water for their chicken activities. The water springs comprised of shallow wells due to high water level in the region and inexpensive systems of shallow well construction. The clime of these regions could be described to be tropical dry forest and savanna environment characterized by marked wet and dry seasons, common of South West Nigeria. It is identified with Precambrian crystalline basement complex rocks. The prevalent rocks represent groups of gneisses and quartzite [6]. The mean annual rainfall is in the range of 1000 to 1250 mm whilst mean annual temperature is about 27°C [6]. The state is bounded in the south by Ogun State, in the North by Kwara State, in the west by Oyo State, and in the East by Ondo and Ekiti States, respectively. This diversity in its boundary delimitations has much positive benefits on the patronage enjoyed by the chicken farms. The sampling stations chosen for this study were regarded suitable because all of them have been in operation for more than twenty-five years. Also, this set of chemicals (As, Cd, Cu, Fe, Pb and Zn) was chosen because they are among the most common contaminants present in well water near chicken waste sites [7]. Furthermore, seasonal analysis of heavy metal contamination of the well waters of the chicken farms chosen was being studied for the first time.

2.2. Sample Collection

Water samples were taken on seasonal basis from both the chicken farms and control sites. For dry season, samples were taken in the months of December, 2014 to March, 2015, whilst for wet season, sampling spanned through the months of July to October, 2015. A total of thirty-two samples were taken employing plastic bucket fastened to a graduated polymer rope. The samples for total metal contents were acidified in the on-site with grade HNO_3, whilst samples for speciation analyses were taken to the laboratory and filtered under pressure via acid washed 0.45 µm millipore nitrate membrane filters.

2.3. Quantification of Some Physicochemical Variables

After collection, the calibrated water quality testing equipment (Ultra meter II^{TM} 6 Psi serial 6207639) was utilized on-site to carry out total dissolved solids (TDS) and conductivity of the water samples, whilst pH was conducted employing calibrated portable pH meter.

2.4. Apparatus and Materials

All glass and propylene ware utilized were first soaked in dilute HNO_3, thoroughly washed with liquid detergent and then rinsed with double distilled de-ionized water and acetone. After that, all the glassware were dried in the oven at 100°C for 24 h, whilst the plastic ware were left to dry ambient temperature and subsequently adopted for water sample collection. All reagent stock solutions comprised 1000 mg$^{/}$L of the metals As, Cd, Cu, Fe Pb and Zn and were of analytical reagent grade provided by BDH.

Table 1. Geographical Locations of the Sampling Sites

Sampling Site	Latitude (N)	Longitude (E)	Elevation (m)
Agboola 1	$07^0 51.540'$	$004^0 16.134'$	318.52
Agboola 2	$07^0 51.563'$	$004^0 16.119'$	311.81
Agboola 3	$07^0 51.587'$	$004^0 16.095'$	311.82
Agboola Control	$07^0 51.767'$	$004^0 16.261'$	328.88
Worgor 1	$07^0 53.961'$	$004^0 17.855'$	353.87
Worgor 2	$07^0 53.948'$	$004^0 17.852'$	357.23
Worgor 3	$07^0 53.927'$	$004^0 17.850'$	356.62
Worgor Control	$07^0 53.857'$	$004^0 17.875'$	362.41
Odunola 1	$07^0 45.196'$	$004^0 30.826'$	317.30
Odunola 2	$07^0 45.243'$	$004^0 30.778'$	323.39
Odunola 3	$07^0 45.290'$	$004^0 30.622'$	313.94
Odunola Control	$07^0 45.223'$	$004^0 30.283'$	323.70

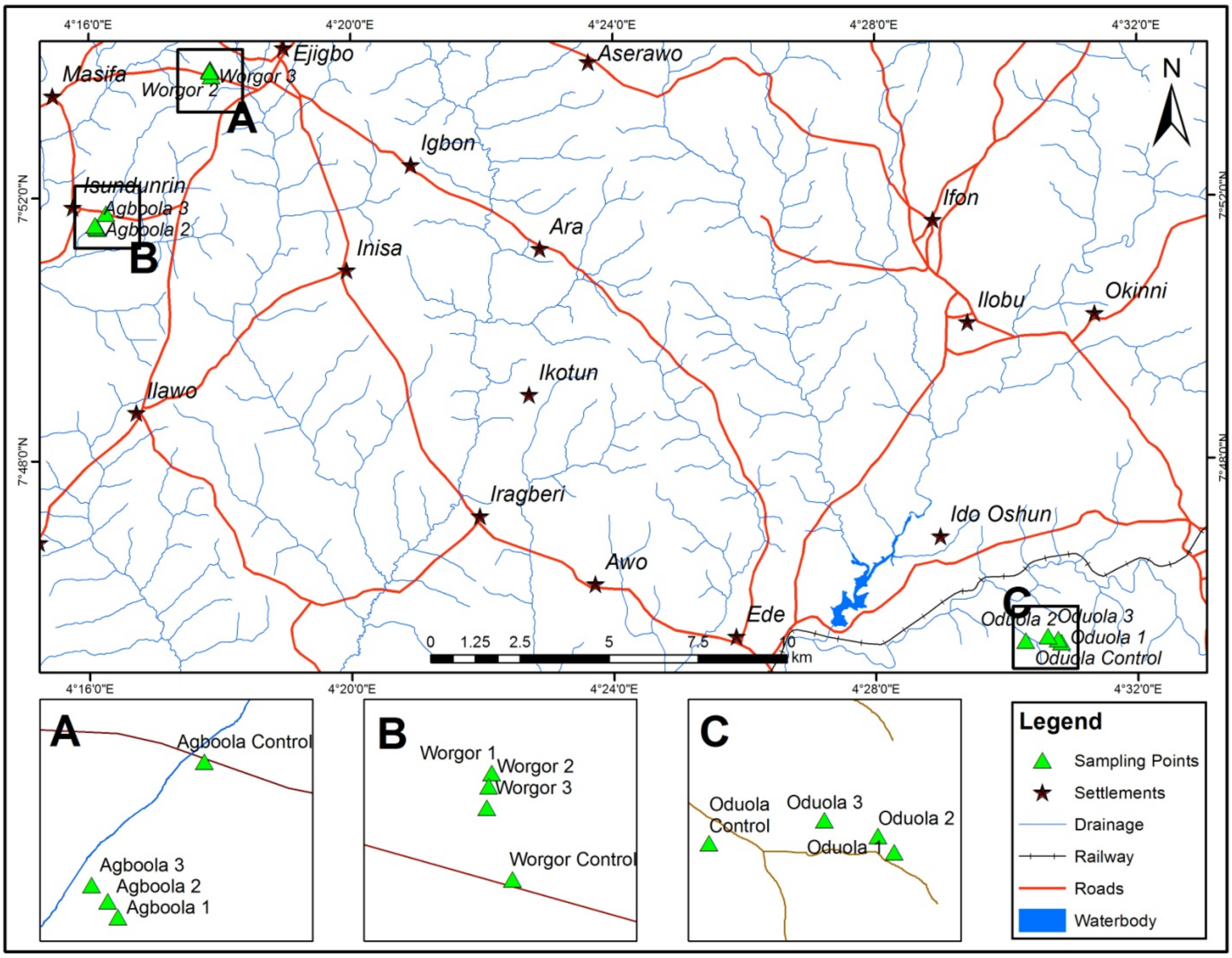

Figure 1. Map of the Study Area Showing Sample Locations

2.5. Digestion of Water Samples for Metals Analysis

The samples were digested utilizing the technique reported somewhere else [5]. Twenty (20) mL of water sample was put in a 100 mL Teflon beaker. Five (5) mL of conc. HNO_3 was included. This was brought to a gentle boiling on a thermostated hot plate. Refilling of content was performed with 1:1 (v/v) HNO_3: $HClO_4$ till total digestion was accomplished. Each digested sample was permitted to simmer down, determinately transferred to a 25 mL titrimetric flask and filled in to the level with distilled water. From this, an aliquot was collected for FAAS analysis. All digestion was conducted on a hot plate in fume hood. A blank analysis was conducted.

2.6. Speciation of Heavy Metals in Water

Utilizing the procedure of Tokalıoğlu et al. [4] which was reworked by Ogunfowokan et al. [8], the Amberlite XAD-16 resin was crushed to increase the surface assimilation and screened to 60-80 mesh. It was cleansed sequentially with methanol, H_2O, 1 M HNO_3 in acetone, H_2O, 1 M NaOH and H_2O. A 400 mg of Amberlite XAD-16 resin suspended was slurry-loaded into a glass column (10 mm i.d x 100 mm length) attached with a glass fiber. Prior to the utilization, the column was prepared with 5-10 mL parts of blank solution (distilled water). This went after the quantification of heavy metals in water samples after sorption on Amberlite XAD-16 resin. This speciation method included three phases: metals in suspended particles, metals bound to humic substances and the free metal ions. The technique is diagrammatically described in Figure 2.

2.6.1. Metals in Suspended Particles

The water samples were screened via a 0.45 µm millipore membrane filter (47 mm diameter). The membrane filters, packed with the suspended particles, were disintegrated in concentrated HNO_3, filtered, vaporized to close to dryness and then taken in a volume of 2 mL with 2 M HNO_3. The quantifications were carried out via FAAS utilizing an injection technique [8].

2.6.2. Metals Bound to Humic Substances

The water samples were screened via a 0.45 µm filter and then the extracts moved through the adsorbent column loaded with Amberlite XAD-16 resin at a rate of flow of 2-2.5 mL min^{-1}. The effluent was set aside so as to carry out the free metal ions. The metal bound to humic substances was then washed out with 1 M HCl in acetone. The eluent was vaporized to close to dryness and the filtrate was disintegrated in 0.7 mL of 2 mol L^{-1} HNO_3.

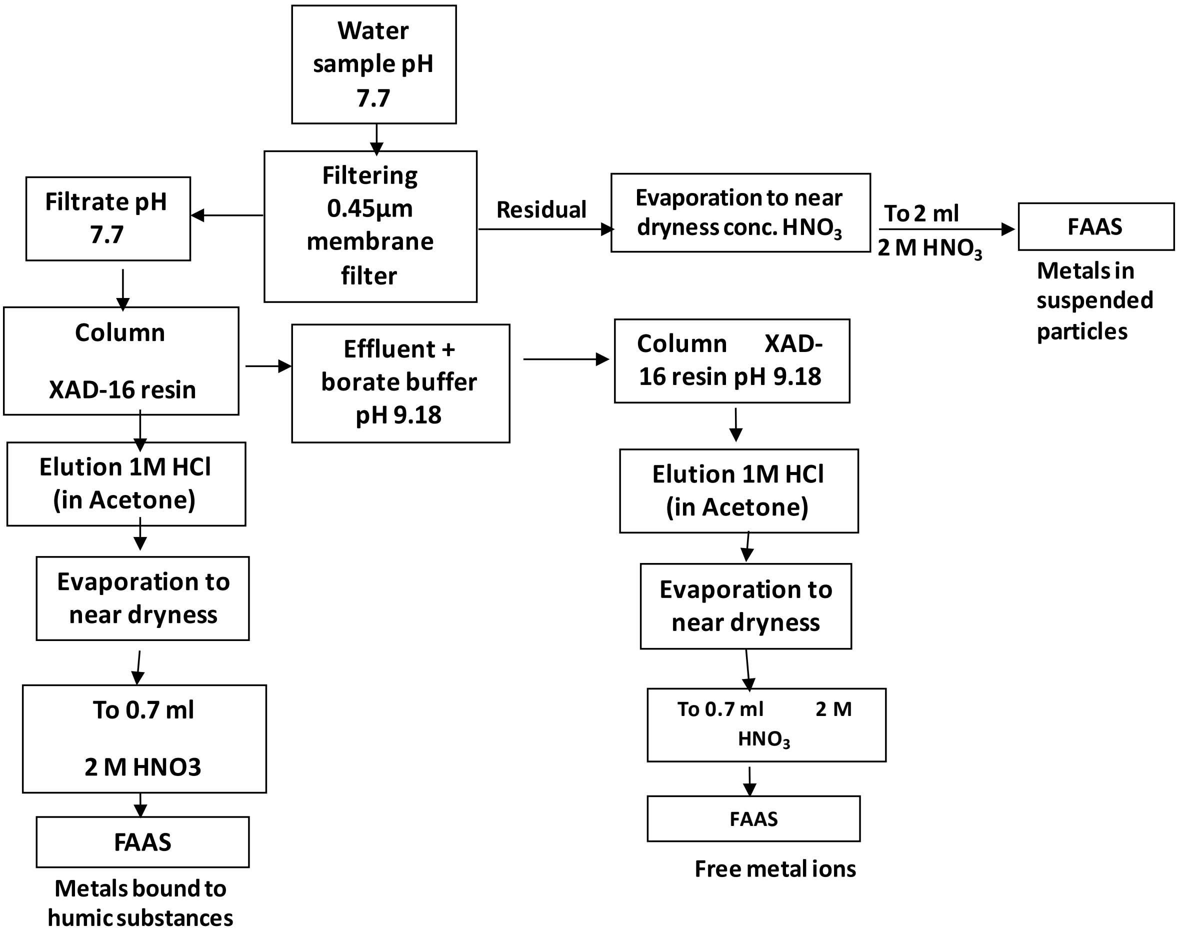

Figure 2. Schematic Representation of Speciation Study of Water Samples (Source: Tokalıoğlu *et al.* (2000))

2.6.3. Free Metal Ions

To carry out the free metal ions in the effluent, a 0.95 g quantity of sodium tetraborate reagent was included to the effluent. The solution was moved through the column packed with Amberlite XAD-16 resin. The trace metals adsorbed on the resin were washed out again with 1 M HCl in acetone. The filtrate was disintegrated in 0.7 mL of 2 M HNO_3 and then free metal ions were assayed using FAAS.

2.6.4. Quality Monitoring Procedures

The analytical quality monitoring conducted involved daily assay of standard and replicate assay of samples and blanks. Owing to the unavailability of standard reference materials for heavy metals in water, spiking technique was employed for the confirmation of the analytical technique. 100 mL double distilled de-ionized water was quantified and transferred into a clean 1000 mL beaker and then spiked with metal standard solutions at fortification values of 15 µg/mL (As, Fe and Fe) and 25 µg/mL (Cd, Cu and Zn). The method as explained in the aforesaid sample treatment, were then reworked. Following the assay the percentage recoveries of the metals were then computed. The detection values per elements were performed utilizing the lowest content of each of the elements that provided the least detectable indicator utilizing FAAS for As, Cd, Cu, Fe, Pb and Zn. Six replicates of each of these contents were made ready and assayed. The detection values were conducted from 3d (d = standard deviation) of the replicate quantifications per of the element [9].

2.7. Data Interpretation

The standard deviation for the metals from three replicate quantifications was conducted. Assessment of the result was performed utilizing the mean values. Analysis of variance (ANOVA) was utilized to express the statistical difference between the means of the heavy metal values found employing SPSS 21.0 for Windows. Coefficient of variation was used to assess the intra and inter site temporal variableness of the metals. A Model PG 990 Atomic Absorption Spectrophotometer provided with a single slot burner was utilized. Analyzes were performed in triplicate.

3. Results and Discussion

3.1. Mean Contents of Total Metals in the Water Samples

The results of the quality control studies revealed satisfactory percentage recoveries for the elements studied in the manner described hereafter: As 98.12%, Cd 83.94%, Cu 90.88%, Fe 92.82%, Pb 89.84% and Zn 98.06%. The calibration contents provided correlation coefficient varying from 0.9856 to 0.9993. The detection values found for the elements assayed were: As 0.20, Cd 0.03, Cu 0.02, Fe 0.50, Pb 0.05 and Zn 0.02 µg/L (Table 2).

Table 2. Values of Percentage Recovery (%R) Study and Calibration Curve Data for Water Sample

Sample	Metal species	Content Spiked (µg/L	Content recovered *	% Recovery	Calibration curve, r^2
Water	As	25	24.53 ± 0.81	98.12	0.9931
	Cd	25	20.99 ± 0.58	83.94	0.9975
	Cu	25	22.72 ± 0.67	90.88	0.9987
	Fe	25	23.21 ± 0.69	92.82	0.9993
	Pb	25	22.46 ± 0.65	89.84	0.9889
	Zn	25	24.52 ± 0.80	98.06	0.9856

The result in Table 3 revealed that the mean value of total As, Cd, Cu, Fe, Pb and Zn in the well water of the chicken farms were statistically higher at $p \leq 0.05$ than the guideline value by World Health Organization (WHO) and National Environmental Standards and Regulations Enforcement Agency (NESREA) for drinkable water.

The total metal burden regarding the aforesaid metals, in µg/mL, was of the order: Control (21.6177) < Worgor (46.0543) < Odunola (50.4667) < Agboola (54.3692) in dry season, and Control (28.4291) < Worgor (58.2661) < Odunola (70.8243) < Agboola (81.4661) in wet season. The increased metal load observed at Agboola farm could be as a result of inappropriate management of manure from the farm, substandard poultry house construction, aging storage facilities used and improper disposal methods used together with possible infiltration from a close by refuse poultry dump over the years, in addition to the geochemical structure of the natural rocks around the well, which might perhaps comprise some of the metals studied. The respective mean total contents levels for both dry and wet seasons ranged between 0.0110–0.0878, 0.0130–0.0900, 0.1124–0.5196, 17.0563-59.4264, 0.021-0.2283 and 3.0420–21.1450 µg/mL for As, Cd, Cu, Fe, Pb and Zn (Table 3).

The content values for the wet season were generally higher than that of dry season. This could be attributed to runoff and percolation from land into the wells during the wet season. Control site reflected the least values of metal levels, while Agboola, Worgor and Odunola farms had the highest levels indicating a direct impact of heavy metals from the chicken farm. Virtually all the metals existed in the water at levels grossly higher than the WHO [10] and NESREA [11] Guideline value for drinkable water (Table 3). This result calls for matter of great concern on the interest of the farmers and chicken workers who may use the water from these wells for household purposes and for feeding their livestock's.

The coefficient of variation (CV) is an effective statistical method that can be utilized to explain the spatiotemporal distribution and variability trends of contaminants in an environmental medium [5]. In the present study, the CV values with a mean value of 9.69, varying from 2.17 in Cu to 11.44 in Zn (Table 3), were not distinctly different from one another. This indicated more or less, a steady rate of input of the metals into the well waters either due to gradual disintegration of the metals from the natural bed rocks or continuing infiltration from the sites of pollution. To put it another way, the factors accountable to the occurrence of these metals in the waters might not be abrupt changes in the man-made activities or clime elements [5].

Table 3. Mean Total Metal Content (µg/mL) of Heavy Metals Found in Water Samples for Four Months each (Dry and Wet Seasons)

				Elements			
Sampling Site	As (Mean ± SD)	Cd (Mean ± SD)	Cu (Mean ± SD)	Fe (Mean ± SD)	Pb (Mean ± SD)	Zn (Mean ± SD)	Total metal burden
Dry Season							
Agboola	0.0650 ±0.003	0.0900 ±0.004	0.2826 ±0.006	38.6763 ±2.830	0.0840 ±0.003	15.1718 ±1.650	54.3692
Worgor	0.0820 ± 0.004	0.0521 ± 0.002	0.2496 ±0.004	32.3546 ±2.580	0.1170 ±0.006	13.1990 ±1.530	46.0543
Odunola	0.0513 ± 0.002	0.0400 ±0.002	0.2790 ±0.005	37.0844 ±2.800	0.0620 ±0.004	12.9500 ± 1.510	50.4667
Control	0.0110 ± 0.001	0.0220 ±0.001	0.1124 ±0.003	17.0563 ±1.930	0.0210 ±0.002	4.3950 ±1.090	21.6177
Range	0.0110-0.0820	0.0220-0.0900	0.1124-0.2826	17.0563-38.6763	0.0210-0.1170	4.3950-15.1718	21.6177-54.3692
Overall Mean ± SD	0.0523 ± 0.0030	0.0519 ± 0.0020	0.2309 ± 0.0050	31.2929 ± 2.560	0.0710 ± 0.0040	11.4288 ± 1.280	43.1269 ± 4.180
CV	5.74	3.92	2.17	8.18	5.63	11.20	9.69
Wet Season							
Agboola	0.0878 ±0.006	0.0590 ±0.003	0.5196 ±0.009	59.4264 ± 4.83	0.2283 ±0.007	21.1450 ±2.61	81.4661
Worgor	0.0599 ±0.004	0.0480 ±0.002	0.3656 ±0.006	43.4020 ±4.55	0.1757 ±0.005	14.2149 ±2.38	58.2661
Odunola	0.0733 ±0.005	0.0130 ±0.001	0.4323 ±0.007	50.4667 ± 4.71	0.2130 ±0.006	19.6260 ±2.57	70.8243
Control	0.0140 ±0.002	0.0228 ±0.002	0.1398 ±0.003	25.1825 ±2.84	0.0280 ±0.003	3.0420 ± 1.83	28.4291
Range	0.0140-0.0878	0.0130-0.0590	0.1398-0.5196	25.1825-59.4264	0.0280-0.2283	3.0420-21.1450	28.4291-81.4661
Overall Mean± SD	0.0588 ± 0.003	0.0357 ± 0.002	0.3643 ± 0.008	44.6194 ± 4.57	0.1613 ± 0.006	14.5070 ± 1.66	59.7465 ± 4.86
CV	5.10	5.60	2.20	10.24	3.72	11.44	8.13
Overall Annual mean ± SD	0.0556 ± 0.004	0.0434 ± 0.002	0.2976 ±0.005	37.9562 ± 2.63	0.1162 ± 0.005	12.9679 ± 2.260	51.4369 ± 4.83
NESREA, (2009) Nigeria	0.010 (P)	0.03	1.00	0.30 (P)	0.010	5.00	
WHO, 2011	0.010	0.03	2.00 (P)	3.00	0.010	3.00	
WA	-	1.00	1400	-	4.00	200.00	

NESREA = National Environmental Standards and Regulations Enforcement Agency; guideline limit for drinking water, WHO (World Health Organization) guideline limit for drinking water; WA (World Average) of trace elements in unpolluted well waters: from Meybeck and Helmer (1989) and Schiller and Boyle (1987). Numbers parenthesis represent (P) – Provisional value; SD = Standard deviation

In proportion to Table 3, the patterns of As, Cd, Cu and Pb follow the same trend with regard to the sample location in both seasons. This implied that the six metals in discussion might have derived from a common source in which the ratio of their form is comparatively steady (Table 5). The topography of Osun metropolis with regard to substratum complex formation and mineral make-up of the soil as derived mostly from crystalline igneous rock disintegration might have a connection to that inclination and most significantly, the elevated content of these metals in the well water which acted as transporting matrix of the minerals in derivative deposition action [5].

3.2. Mean Metal Speciation in Well Water

The data of mean metal speciation in the well water of the chicken farm are presented in Table 4 for dry and wet seasons.

3.3. Metals in Suspended Particle Phase

The phase of element connected with particles > 0.45 µm is referred to as the suspended particles and is the quantity of elements extracted during centrifugation and filtration. The mean total content levels varied between 0.0014-0.0203, 0.0012-0.0139, 0.0098-0.1327, 3.5907-13.0046, 0.0025-0.0482 and 0.0375-3.5503 µg/mL for As, Cd, Cu, Fe, Pb and Zn, respectively as can be found in Table 4. The content values for wet season were generally higher than that of dry season. This could be ascribed to chicken runoff from land into the hand-dug well water during the wet season. Consequently, the suspended particulate might be the oxides of the metals. The Fe and Zn contents in water samples from the wells were statistically higher than the values for the other metals in both dry and wet seasons.

The elevated levels shown by Fe and Zn indicated that there was deposition of Fe and Zn comprising particulates on the farm soils surrounding the wells especially during precipitation. In addition, farming activities around the wells could have contributed immensely to the detected increased levels of Fe and Zn contents since these metals can occur as contaminants in chicken feeds concentrates and in metal-based pesticides and manure.

Other researchers [12,13] have reported that Fe and Zn emission from chicken farms produced elevated contents of elements in chicken farm well water, surface water, soil and vegetation due to extensive use of antibiotics, drugs, anti-oxidants, grits, minerals, anti-fungal and pre-mix, not only to treat or avert microbial infection in animals, but are also commonly utilized to promote more rapid growth in livestock industry of USA and Nigeria [12]. The content of Fe and Zn in all the wells studied might also have been influenced by the effluent generated from the chicken house and inappropriate disposal of animal wastes and abandoned livestock facilities over the years on the sites.

The values reflected by As, Cd, Cu and Pb were similar. It is possible that the low cost poultry houses roofed with metallic alloys of Cd, Cu and Pb corrugated sheets could have added to their contents obtained. It was also possible that As, Cd, Cu and Pb might have been leaked into the surrounding ecosystem from the chicken activities since the pH of the wells analyzed were marginally within slightly acid to slightly alkaline range of 6.38-7.60. As regards Cd, input from chicken industry contamination of chicken farm soil and vegetation by Cd is usually less abnormal than that of Fe and Zn [12].

The As, Cd, Fe, Pb and Zn more than the Nigeria and WHO quality guidelines value of 0.010, 0.030, 0.010 and 5.00 µg/L, respectively as revealed in Table 3 [10,11]. The value revealed by Cu is well within the water quality guidelines value of NESREA and WHO. This evaluation is essential since the hand-dug wells are utilized daily by the farmers, chicken workers and local inhabitants for household purposes, like bathing, laundry and dishes, drinking and cooking, mouth brushing, preparing concentrate, antibiotics, feeding their livestock, washing of tools, cleaning of the equipment, cleaning of the housing units of animals, irrigation, washing or cleaning of animals and dipping or delousing of animals without further treatment.

The water quality variable for the water sample utilized for the speciation analyzes is presented in Table 5 from where it can be observed that there was no statistical difference between the dry and wet season pH contents. Also the pH contents from the sampling points were more or less the same (6.34-7.60). Nevertheless, the conductivity and total dissolved solids contents for the dry season were statistically more than that of wet season. During the dry season, percolation from poultry waste might have added high amount of suspended particulates substances into the water ecosystem.

By and large, it could be noticed that all metals revealed low contents in the particulate phase. This could be due to low predominant states of metals, like As, Cd, Cu, Fe, Pb and Zn that were commonly adsorbed or co-precipitated as hydroxides of basic salts [3]. This phase is observed to contain low bioavailability.

3.4. Metal Bound to Humic Substances

The bioaccumulation or complexation activity is the major source by which trace metals become closely related to organic substance like living organisms, detritus, and so on [8,14]. In aquatic ecosystems, an organic substance likely to have high level selectivity for individual ions in comparison to monovalent ions into organic substance. In organic matter phase, metallic contaminants bound to this phase are supposed to remain in the soil for longer terms but may be immobilized by disintegration activity [8]. The mean total content values varied between 0.0058-0.0412, 0.0035-0.0236, 0.0643-0.2321, 4.8670-16.0665, 0.0033-0.0763 and 0.5596-6.9173 µg/mL for As, Cd, Cu, Fe, Pb and Zn, respectively (Table 4). Arsenic and Cu are available more in the humic phase. This signified that As and Cu might have present in state of metal complexes that did not separate on the column and these are more probable to connect with organic substances and thus, not available.

Léonard [15] observed the speciation and dissolution of As and Cu in spring water suspension to be caused by pH and oxidation-reduction potential. Under oxidizing environment, breakdown of organic matter can bring about a production of soluble trace metals of As and Cu bound to this constituents. The result of As and Cu corresponds with the data of other investigators [16,17]. Also, As and Cu are known to available as polyvalent ions and tie strongly to negative charged sets of organic substance. It has also been revealed that As and Cu in specific tie very strongly to humic substances [18]. It seems persuasive that production of water sample column raises the complexation of metals. The higher relationship of As and Cu in the moderately organic substances phase are as a result of the higher stability of their As and Cu-oxides than Cd, Fe, Pb and Zn-oxides [19]. Once more, this phase is less bioavailable in the ecosystem.

3.5. Free Metal Ions

The elements held by free metal ion complexes (< 0.45 µm) are those left in the sample after ion exchange and those that include the utilization of sodium tetraborate reagent to conduct the water sample. The content of Cd, Fe, Pb and Zn were higher in the free metal ion phase, signifying that a comparatively larger part of these heavy metals were bonded to free metal ion. From another point of view, they revealed an almost constant distribution trend in the free metal ion phase in both dry and wet seasons. The free metal ions (substances kept on Amberlite XAD-16 resin glass column) would comprise hydrated metal ions and metal complexes that separated on the glass column. Since the first highest amount of Cd, Fe, Pb and Zn were in this phase, they were thus bioavailable. As a result, these may present a health risk to humans, livestock and aquatic organisms taking into account the health possible effects of the metals. In the present study, the content of metals that can be observed to be bioavailable to the aquatic ecosystems varied between 0.0026-0.0249, 0.0057-0.0512, 0.0123-0.1538, 6.3280-24.3553, 0.0086-0.1073 and 2.2178-10.9546 µg/mL for As, Cd, Cu, Fe, Pb and Zn, respectively (Table 4). These contents were statistically more than those observed on the toxicity of these metals on algae [17].

Table 4. Mean Heavy metal contents (µg/mL) in the Varied Speciation Phases in the Poultry Farm Well Water in the Study Sites

Metal		Sampling points										
		December- March (Dry)				July – October (Wet)						
	Phases	A	B	C	D	A	B	C	D	Range	Overall Mean ± SD	CV
As	Metal in Suspended Particles	0.0123±0.002	0.0193 ± 0.003	0.0086 ±0.002	0.0021 ±0.001	0.0203 ±0.003	0.0063 ±0.001	0.0131 ± 0.001	0.0014 ± 0.001	0.0014-0.0203	0.0105 ± 0.003	28.51
	Bound to Organic Substances	0.0348 ± 0.003	0.0387 ± 0.004	0.0326 ± 0.003	0.0058 ±0.002	0.0412 ±0.003	0.0261 ± 0.002	0.0342 ± 0.001	0.0058 ± 0.002	0.0058-0.0412	0.0274 ± 0.005	18.25
	Free Metal Ions	0.0149 ±0.002	0.0223 ± 0.003	0.0123 ±0.002	0.0027 ±0.001	0.0249 ±0.002	0.0124 ± 0.002	0.0129 ±0.001	0.0026 ± 0.001	0.0026-0.0249	0.0131 ± 0.004	30.54
Cd	Metal in Suspended Particles	0.0113 ± 0.003	0.0088± 0.001	0.0012 ±0.001	0.0021 ± 0.001	0.0139 ±0.002	0.0120 ± 0.002	0.0025 ±0.001	0.0058 ± 0.002	0.0012-0.0139	0.0072 ± 0.002	27.78
	Bound to Organic Substances	0.0236 ± 0.003	0.0112 ±0.002	0.0128 ± 0.002	0.0035 ±0.002	0.0186 ±0.001	0.0141 ± 0.002	0.0043 ± 0.002	0.0075 ± 0.002	0.0035-0.0236	0.0120 ± 0.003	25.00
	Free Metal Ions	0.0512 ± 0.004	0.0318 ±0.003	0.0253 ± 0.003	0.0143 ±0.002	0.0256 ±0.002	0.0223 ± 0.003	0.0057 ± 0.002	0.0093 ± 0.003	0.0057-0.0512	0.0232 ± 0.006	25.86
Cu	Metal in Suspended Particles	0.0213 ± 0.002	0.0247 ±0.002	0.0325 ± 0.002	0.0098 ±0.003	0.1327 ±0.004	0.0699 ± 0.002	0.0947 ± 0.003	0.0392 ± 0.002	0.0098-0.1327	0.0531 ± 0.008	15.09
	Bound to Organic Substances	0.1965 ±0.005	0.1801 ±0.004	0.0998 ±0.004	0.0775 ±0.003	0.2321 ± 0.003	0.1656 ± 0.003	0.2089 ± 0.002	0.0643 ± 0.002	0.0643-0.2321	0.1531 ± 0.030	19.60
	Free Metal Ions	0.0509 ± 0.003	0.0426 ±0.002	0.0446 ±0.003	0.0123 ±0.002	0.1538 ±0.003	0.1290 ±0.003	0.1290 ± 0.004	0.0356 ± 0.001	0.0123-0.1538	0.0744 ± 0.004	5.38
Fe	Metal in Suspended Particles	6.1330 ± 1.060	3.5907 ± 1.020	7.1300 ±1.680	3.9590 ±1.078	13.0046 ± 2.932	5.7335 ± 1.365	8.0534 ± 1.724	6.1300 ± 1.625	3.5907-13.0046	6.7168 ± 1.750	26.05
	Bound to Organic Substances	10.2487 ± 2.040	12.5168 ± 2.086	11.2256 ±2.052	4.8670 ±1.136	16.0665 ±3.060	9.9075 ± 1.850	15.2485 ± 3.850	8.2253 ± 1.930	4.8670-16.0665	11.0382 ± 2.400	21.74
	Free Metal Ions	19.5577 ±2.460	15.0751 ± 2.420	18.3263 ±2.600	6.3280 ±1.262	24.3553 ± 3.795	19.6197 ± 3.830	22.0277 ± 4.080	11.3210 ± 2.190	6.3280-24.3553	17.0764 ± 3.430	20.09
Pb	Metal in Suspended Particles	0.0126 ±0.002	0.0267 ± 0.002	0.0163 ±0.002	0.0025 ±0.001	0.0316 ±0.002	0.0396 ± 0.002	0.0482 ± 0.003	0.0043 ± 0.001	0.0025-0.0482	0.0227 ± 0.004	17.62
	Bound to Organic Substances	0.0184 ±0.003	0.0398 ±0.003	0.0187 ±0.003	0.0033 ±0.001	0.0763 ± 0.003	0.0567 ± 0.002	0.0683 ± 0.003	0.0098 ± 0.002	0.0033-0.0763	0.0364 ± 0.006	16.48
	Free Metal Ions	0.0318 ±0.003	0.0463 ±0.004	0.0264 ±0.002	0.0086 ±0.002	0.1073 ±0.002	0.0786 ± 0.003	0.0951 ± 0.004	0.0136 ± 0.002	0.0086-0.1073	0.0510 ± 0.007	13.73
Zn	Metal in Suspended Particles	2.3339 ±0.680	3.5503 ± 0.753	1.6928 ±0.580	0.9188 ±0.091	3.3019 ±0.740	3.5503 ± 0.810	3.4426 ± 0.760	0.0375 ± 0.003	0.0375-3.5503	2.3535 ± 0.650	27.62
	Bound to Organic Substances	4.2028 ±1.290	4.1736 ± 1.220	3.0804 ±1.133	1.0806 ±0.976	6.9173 ±1.850	4.1736 ± 1.233	5.6426 ± 1.740	0.5596 ± 0.080	0.5596-6.9173	3.7288 ± 0.860	23.06
	Free Metal Ions	6.2787 ±1.421	5.3127 ±1.383	5.8528 ±1.346	2.3955 ±0.986	10.9546 ± 2.490	6.3127 ± 1.543	9.3364 ± 2.316	2.2178 ± 1.030	2.2178-10.9546	6.0827 ± 1.720	28.28

A, Agboola farm; B, Worgor farm; C, Odunola farm; D, Control; SD = Standard deviation.

Table 5. Mean Chemical Variables of Well water Samples for Dry and Wet Seasons (µS/cm and mg/L)

Sampling Site	Conductivity (**µS/cm**)	pH	TDS (**mg/L**)	Total Alkalinity (**mg/L**)
Dry Season				
Agboola	623.57 ± 21.88	6.73 ± 0.07	386.33 ± 20.41	221.10 ± 3.46
Worgor	282.25 ± 10.36	6.38 ± 0.05	178.20 ± 5.89	159.15 ± 2.86
Odunola	381.55 ± 12.75	6.60 ± 0.06	245.32 ± 6.62	168.67 ± 2.93
Control	201.42 ± 9.82	6.97 ± 0.08	126.62 ± 2.50	114.13 ± 1.91
Overall Mean ± S.D	372.20 ± 15.85	6.67 ± 0.07	234.11 ± 14.86	165.76 ± 2.91
CV	4.26	1.05	6.35	1.76
Wet Season				
Agboola	589.07 ± 18.92	7.41 ± 0.08	310.43 ± 0.93.	201.67 ± 1.48
Worgor	180.97 ± 4.16	6.40 ± 0.06	112.87 ± 0.91	150.07 ± 1.08
Odunola	358.20 ± 3.80	6.65 ± 0.07	229.70 ± 0.92	161.67 ± 1.11
Control	173.67 ± 2.13	7.60 ± 0.09	108.40 ± 0.84	101.33 ± 1.02
Overall mean ± S.D	325.48 ± 10.17	7.02 ± 0.09	190.35 ± 0.98	153.69 ± 1.09
CV	3.12	1.28	2.05	0.71
Annual mean	348.84 ± 11.18	6.85 ± 0.08	212.23 ± 12.80	159.73 ± 2.06
WHO, (2011)	1200	6.5-8.5	0-1000	200
NESREA, (2009)	1000	6.5-8.5	500	200
SON, (2006)	1000	6.5-8.5	500	200

Table 6. Analysis of Variance of Total Metal Analysis

Source of Variance	Sum of Squares	Degree of Freedom	Mean Square	$F_{calculated}$	p(same)
Between Groups	18614	5	3722.80	154.10	1.871E-10
Within Group	289.922	12	24.1602		
Total	18903.90				

The data for the speciation of As, Cd, Cu, Fe, Pb and Zn in water appear to be in comparison to those of some other investigators who accounted that the highest species of Cd, Fe, Pb and Zn was available in the free aquo ion. The resemblance is as a result of the water chemistry of the chicken farm well water of Osun State with annual mean pH (6.85) and alkalinity (159.73 mg/L) contents. Akoto and Abankwa [20] found that over 50% of As, Cd, Cu, Fe, Pb and Zn occurred as a free hydrated ion for water of lower pH (7.2) and alkalinity (126 mg $CaCO_3$/L) contents. The speciation of Cd in the water column was in comparison to that of Fe, Pb and Zn. Soluble Cd was found mostly as a free ion (Cd^{2+}). This is as a result of the soft acid nature of Cd and the hard base nature of carbonate ion [21] causing a lower chemical attraction to suspended particles and humic substances species, and as a result, the likelihood to produce organic complexes may be low [22].

Lastly, the element contents in the free metal ion samples were higher than in the humic substances and suspended particles unless in As and Cu instances. Seasonal differences in the amount of suspended particles, bound to humic substances and metal free ions in the water samples might have occurred because the inflow and precipitation rate of the metals, suspended particles and organic materials usually count on the seasons.

4. Conclusions

A three stage method by Tokalıoğlu et al. (2000) which was reworked by Ogunfowokan et al. (2013) was employed to fractionate heavy metals in the water samples of chicken farms to suspended particles, bound to humic substances and free metal ions phases. The contents of mean heavy metals in water speciation followed the same order in both dry and wet seasons: Free metal ions > Bound to humic substances > Suspended particles. The work also revealed that a large quantity of Cd, Fe, Pb and Zn found in water samples was mainly bioavailable since the elements were largely inherent in free metal ions. This signified that they could present health risks to humans (chicken workers) and chicken livestock organisms observing the health likely consequences of these metals. Moreover, the distributions of elements amongst the different phases were present to be comparatively related in all the four sites unless at the control site signifying that the results could perhaps be characteristic of more samples than those analyzed in this work. Periodic monitoring of well water quality, abrogation of unhealthful chicken waste disposal practices and introduction of modern poultry techniques are recommended.

Statement of Competing Interests

The authors declare that they have no competing interests.

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