Speciation Partterns of Some Selected Heavy Metals in the Soil along the Effluent Channels of Juhel Pharmeutical Industry, Awka, Anambra State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author PACO designed the study and wrote the protocol while author HOA performed the statistical analysis and wrote the first draft of the manuscript. Authors AGE and UCU managed the analyses of the study. Author AGE managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The speciation of some selected heavy metals in soil samples collected along the effluent channels of JUHEL Pharmaceutical industry, Awka was investigated. The pH of the soils had average value of 7.53 and moisture content of 17.96%. The results revealed total metal concentration range of 0.13 mg/kg to 16.49 mg/kg among the metals –Cd, Cr, Pb, Ni, Cu, Co, Zn,Mn, Fe and Ca. Cadmium (Cd) had the lowest metal concentration with a value of 0.13 mg/kg and iron (Fe) had the highest concentration with a value of 16.49 mg/kg. The observed trend for the total metal concentrations was: Fe > Ca > Mn > Pb > Cr > Co > Cu > Ni > Zn > Cd. The computed bioavailability of the metals, for the industry, followed the trend: Ca > Pb > Cu > Cr > Mn > Ni > Cd > Co > Zn > Fe. Metals speciation of the soil samples showed that the highest and lowest partitions were found in the residual and water soluble for the metals Fe, Mn, Pb, Cr, Cu, Ni, Co and Zn,

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respectively except for Ca which had its highest concentration in the carbonate bound partition, and the lowest concentration in the water soluble fraction. The highest fraction for Cd was found in the residual and Fe-Mn oxide (reducible) forms, with the water soluble fraction having the lowest Cd concentration. Comparison with NESREA standard also revealed low contamination level of the metals, and there was no mean significant difference to conclude that the pollution level of the environment was alarming.

1. INTRODUCTION

In recent years, heavy metal pollution has become one of the most serious environmental problems. Presence of heavy metals even in traces is toxic and detrimental to both flora and fauna. With the rapid development of many industries, wastes containing metals are directly or indirectly being discharged into the environment causing serious environmental pollution and even threatening human life [1]. The quality of environment is a matter of serious concern, especially today that the consequences of human intervention are already evident. Although environment is extremely valuable for people and other organisms living in it, it is also endangered due to human activities that are continuously ruining it [2], and the activities of Juhel Pharmaceutical industry is not an exception. The activities in this industry include the use of different kinds of chemicals in the manufacture of drugs and plastics. Petroleum products are equally used for different purposes: transportation, generation of electricity etc; all of which may get contaminants into the environment by some other routes other than industrial effluent discharge.

Metals and metalloids due to their extensive use represent an important fraction of the pollutants released in air, soil and water. As human activities impacts upon the environment, metal contamination issues are becoming increasingly common [3]; and metals that used to exist in traces in the environment due to natural sources, have become hazardous nowadays, due to their elevated concentration through human contributions [2]. For example, in Zamfara State of Nigeria, the mass Lead poisoning from mining activities, which was discovered in March 2010, continued to affect villagers in three local government areas: Anka, Bukkuyum and Maru. And while the full scale of the problem was still not fully determined, a survey carried out by the US Centers for Disease Control and Prevention (US CDC) at the request of the Nigeria Federal Ministry of Health revealed at least 43 villages in Zamfara State where there are confirmed cases of Lead poisoning (blood Lead concentration >10 µg/dL), and with over 400 children dead. In atleast seven of these villages, there are children who need chelation therapy (blood Lead concentration >45 µg/dL). This was in addition to the seven villages that had been remediated. With the combined effects of removal from Lead exposure and chelation and other supportive therapy, the mortality rate among exposed children in seven remediated villages has dropped from 43% in 2010 to 1% in 2011 [4].

Sources such as atmospheric deposition, waste disposal, fertilizer application and wastewater in agricultural land constitute the major anthropogenic inputs. Generally the distribution of heavy metals is influenced by the nature of parent materials, climatic conditions, and their relative mobility depending on soil parameters, such as mineralogy, texture and classification of soil [5]. Some physicochemical properties of soils such as pH and CEC are important parameters that control the accumulation and the availability of heavy metals in the soil environment. The main objectives of this paper are (1) to determine the range and distribution of heavy metals concentrations in the soils of the effluent channels of Juhel industry (2) to assess the principle physical and chemical parameters, affecting heavy metals distribution in the soil and (3) to evaluate the anthropogenic contribution.

2. EXPERIMENTALS

2.1 List of Apparatus

The following instruments were used: pH meter (Jenway 3505 model), centrifugation machine and AAS machine (Analyst 200 flame spectrophotometer).

2.2 List of Reagents

All reagents used were made by Sigma-Aldrich and were all analar grade reagents. No further purification was done.

2.3 Preparation of Reagents [6]

2.3.1 1 M Sodium Acetate (CH₃COONa)

35 g of NaOAc powder was weighed into 500 ml volumetric flask, dissolved with deionized water and made up to the mark. A pH of 5 and 8.5 respectively were obtained by adjustment with Acetic acid.

2.3.2 0.04 M Hydroxylamine Hydrochloric Acid (NH₂OH.HCl) in 25% v/v Acetic acid

1.39 g of $NH_2OH.HCl$ was weighed into a 500 ml volumetric flask, dissolved with 25% v/v HOAc (i.e. 75 ml Acetic acid in 175 ml of water) and made up to mark with deionised water.

2.3.3 0.02 M HNO₃

With a percentage purity of 69 and specific gravity of 1.42, some 1.4 ml of the stock HNO_3 was taken and dissolved to a 1000 ml flask of deionised water and made up to mark.

2.3.4 3.2 M Ammonium Acetate CH₃COONH₄ in 20% v/v HNO₃

64.8 g of NH_4OAc was weighed into a 250 ml flask, dissolved with 20% v/v HNO3 and made up to mark.

2.3.5 30% H₂O₂

2 ml of a 35% H_2O_2 commercial stock was dissolved in 98 ml of deionised water to obtain a 100ml solution of 30% H_2O_2. A pH of 2 was obtained by adjustment with HNO_3.

2.4 Sampling and Sample Pretreatment

Juhel Pharmaceutical Industry is located along Awka-Enugu express way. It is the only manufacturing industry within the vicinity. Soil samples were collected with plastic containers from the effluent channels and packed in polythene bags and taken to laboratory immediately for digestion and sequential extraction fractionation. Partial aeration was performed and appropriate particle size was obtained using 1.5 mm nylon sieve. In collecting the samples, a distance of 20 meters apart was maintained, and a total of 6 soil sample points were taken along the industry discharge channels. Samples were collected in rainy season (April to August). Fig. 1 is the map of the industry location.

2.5 pH Determination

Approximately 5 g of the soil samples was taken and equilibrated for 30 minutes in thoroughly washed and dried beakers containing 20 ml of deionised water. The pH values were determined using pH meter, Jenway 3505 model, and recorded.

2.6 Moisture Content

Approximately 5 g of the wet soil samples was taken in a beaker and placed in the oven for 24 hrs. The weights taken, W_1, weights after heating, W_2, moisture content and percentage moisture were recorded.

2.7 Sample Digestion for Total Metal Analysis

Approximately 2 g of the soil sample was placed into a Teflon container and digested by addition of 5 ml HF and 5ml aqua regia, and heating on a water bath for one and half hours. After cooling, fresh volumes of HF and aqua regia, 5 ml each, was added and digested again for another one and half hours heating on the water bath. Finally, 20 ml volume of saturated boric acid (H_3BO_3) was added on cooling after the final digestion process to complex the residual hydrofluoric acid (HF) which would otherwise attack glass wares. Filtration of the samples was done with Whatman No.1 filter paper and the solution made up to 50 ml mark.

2.8 Sequential Extraction of Heavy Metals

2.8.1 Water soluble fraction

To 2 g of soil sample, 10 ml of deionised water was added in a 50 ml Teflon centrifuge tubes and agitated for 30 minutes before centrifugation and decantation into the sample bottles for storage. Subsequent series of washing with 10 ml of deionised water was done to make up to 50 ml mark of the sample bottles.

2.8.2 Exchangeable fraction

To the residue from previous leach, 8ml of 1M sodium acetate (NaOAc pH 8.5) was added and agitated for one hour at room temperature before centrifugation and decantation. The residue was washed with deionised water and 4 ml of aqua regia added to the liquid sample taken for analysis before making up to 50 ml mark of the sample bottles with deionised water.
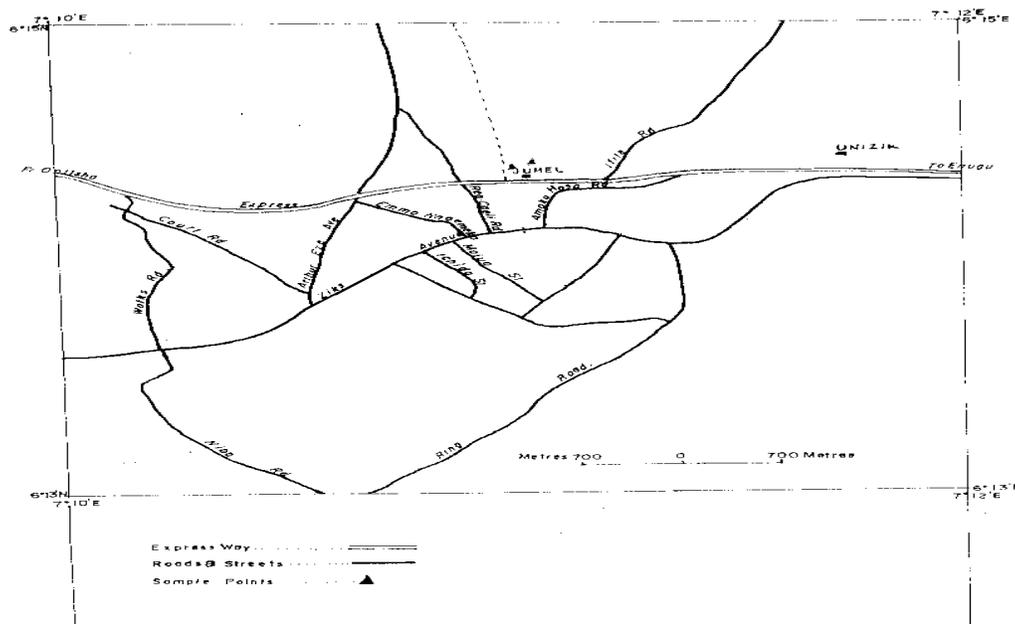
Fig. 1. Map of Awka showing JUHEL location and sampling point

2.8.3 Carbonate-bound fraction

To the residue from previous leach, fresh 8 ml 1 M sodium acetate solution (adjusted to pH 5 with acetic acid) was added and agitated for one hour at room temperature before centrifugation and decantation. The residue was washed with deionised water while 4 ml aqua regia was added to the liquid sample taken for analysis and made up to mark with deionised water.

2.8.4 Fe-Mn oxide fraction (reducible)

To the residue from previous leach, 20 ml 0.04 M $NH_2OH.HCl$ in 25% v/v acetic acid was added and agitated periodically in boiling water bath for 5 hours. The residue was washed with deionised water after centrifugation and decantation. The liquid sample obtained for analysis was added 4 ml of aqua regia and made up to the mark.

2.8.5 Organic and sulfide fraction (oxidizable)

To the residue from previous leach, 3 ml 0.02 M HNO_3 and 5 ml of 30% H_2O_2, which has been adjusted to pH 2 with HNO_3, was added and agitated periodically in hot water bath (85°C) for 2 hours. This was followed by addition of 3 ml H_2O_2 (pH 2) and periodic agitation in the hot water bath for another 3 hours. After cooling to room temperature, 5 ml of 3.2 M ammonium acetate in 20% v/v HNO_3 was added finally and

agitated at room temperature for 30 minutes before centrifugation and decantation to obtain the liquid sample for analysis. The residue was washed with deionised water and 4 ml aqua regia was added to the liquid sample for analysis before making it up to mark in the sample bottles.

2.8.6 Residual fraction

To the residue from previous leach, 5 ml HF and 5 ml aqua regia was added to digest it. This was heated in a hot water bath for 2 hours. Centrifugation was followed by decantation and deionised water was used to make up to the mark.

All through the exercise, double portions of the normal reagent volumes were used for the 2 g of soil fractionated. And all the stored supernatant solutions obtained for all the fractions, as well as a blank, were instrumentally analyzed for the selected metals concentration using *Analyst 200* model of AAS machine

3. RESULTS AND DISCUSSION

3.1 Moisture Content and pH of Soil Samples

The moisture content and the pH of all the soil samples used for this study are presented in Table 1.

The average moisture content (Table 1) of the soil sample from the effluent channels of JUHEL pharmaceutical industry was 17.96% while the pH value was 7.53.

With a pH value of 7.5, it may be said that the soils are between neutrality and slightly alkaline. The metals bound to acid soluble or carbonates may remain attached to the soil pending pH change to acidity, as imminent change in pH of the soil due to an external influence, or changes in redox or other favourable conditions, would increase the level of some metal concentration in the soil solution/moisture available for absorption into biota.

The influence of rain and steady inflow of waste water contributed to the significant moisture content of the soil; hence with favourable conditions, absorption of the metals together with the moisture into biological tissue would take effect; or the evaporates may well transport the metals through the atmosphere and then breathed in air.

3.2 Total Metal Concentrations in Soil

The results from the total digestion of soil to determine its total metal concentration is presented in the Table 2. The results revealed a concentration range of 0.13 – 16.49 mg/kg of the soils among all the metals. Fe > Ca > Mn > Pb > Cr > Co > Cu > Ni > Zn > Cd, was the observed trend for the total metal concentration.

3.2.1 Cadmium

The speciation of Cd in the soil is presented in Table 3.

The speciation result for Cd at JUHEL, as shown (Fig. 2) indicated that for the whole Cd content of the soil, a quite significant amount of the metal was in the residual fraction, while the rest were associated with the exchangeable and carbonate, as well as a very small fraction in the organic/sulfide fraction. The low percentage bioavailability of the metal directly pointed to a strongly bound Cd with a very slight mobility and availability. The observed trend for the metal speciation was F6 > F3 = F4 > F5. It was not detectable in F1 and F4.

3.2.2 Chromium

The speciation of Cr is presented in Table 4.

The speciation of Cr at JUHEL as shown (Fig. 3), simply indicated that the various forms of

association of the metal in the soil vicinity was evenly distributed; and may be interpreted as partially strongly bound, and partially bioavailable. The little fraction that appeared in the water soluble vividly showed the likely mobility of the metal around the soil vicinity of the industry.

Again, the fractions bound to organic/sulfide and Fe-Mn oxide went to show that any slightest favorable condition would increase the percentage bioavailability of the metal in the soil vicinity. There was slight significant anthropogenic contribution.

3.2.3 Nickel

The speciation of Ni is as presented in Table 5 and Fig. 4.

Ni speciation at JUHEL had its highest concentration in the residual fraction. That was followed by the exchangeable and carbonates fractions, with a smaller concentration in the organic/sulfide and Fe-Mn oxide. The metal was very slightly detectable in the water soluble fraction. Therefore it may be said that Ni, as well as any other metal detected in the water soluble fraction, could be moving with runoff water and would be more so at any slightest favorable constraint on the exchangeable and carbonate forms as these forms accounts for the metal's anthropogenic contribution and its bioavailability.

3.2.4 Lead

The speciation result of Pb is as presented in Table 6 and Fig. 5.

Pb concentration in the soil of JUHEL effluent dischatge channel appears low. The highest concentration of Pb found in the soil of JUHEL, as presented in the table and graph, was found in the residual, followed by the carbonate and the exchangeable. A trace amount was found in the water soluble, and the remaining was associated with the Fe-Mn oxide and the organic/sulfide. The percentage bioavailability of the metal was quite tangible, up to 44.32% and that was a vivid indication of possible anthropogenic contribution. The observed trend for the metal speciation was F6 > F3 > F2 > F4 > F5 > F1.

3.2.5 Cobalt

The result of the speciation of Co is outlined in Table 7 and shown pictorially in Fig. 6.

The highest concentration of Co at JUHEL was observed in the residual form, which may not necessarily stir concerns towards environmental risk; and that was followed by the little concentrations obtained in the exchangeable and the carbonates. Although the bioavailability of the metal, as made up by the first three fractions in the Table, was considerably low, its detection in all the fractions showed the distribution of the metal as well as its mobility picture. The observed trend for the metal speciation was F6 > F3 > F2 > F4 = F5 > F1.
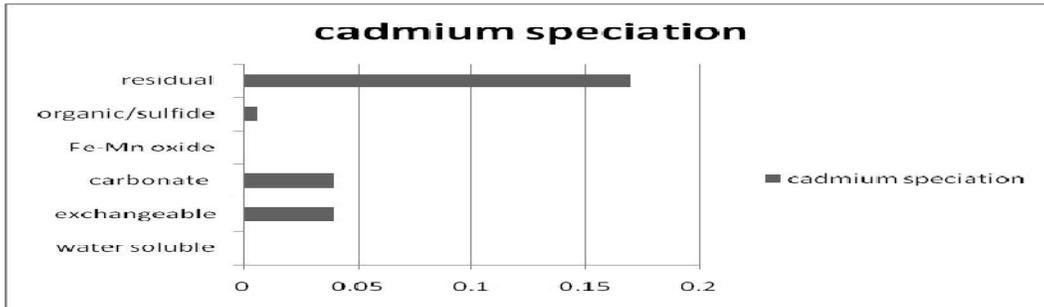
Fig. 2. Graphical presentation of cadmium speciation
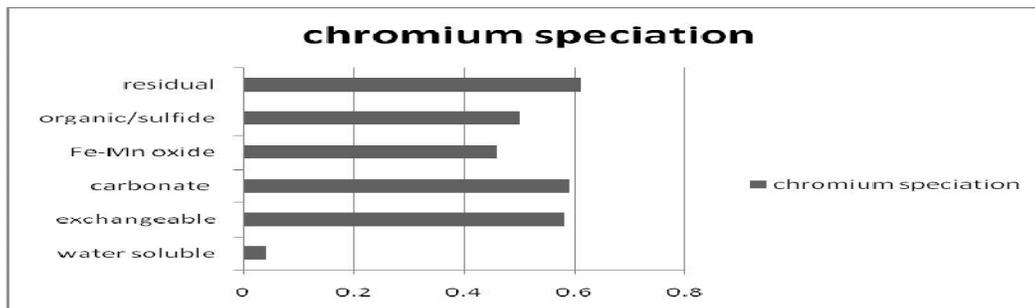
Fig. 3. Graphical presentation of chromium speciation
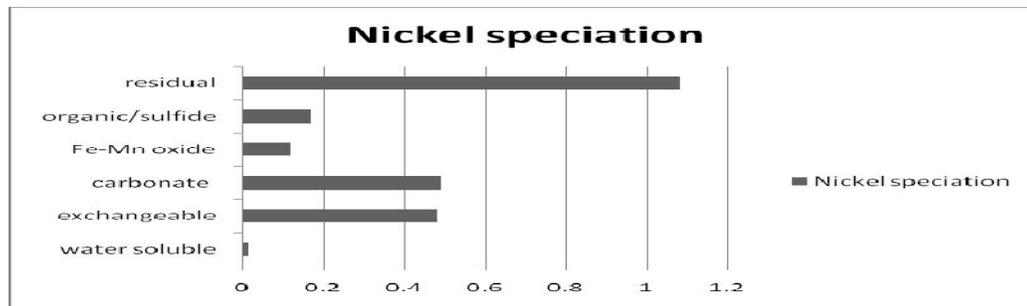
Fig. 4. Graphical presentation of nickel speciation

Table 1. Moisture content and pH values

Sample code.	Weight taken (W_1) (g)	Weight after heat (W_2) (g)	Moisture content (g)	% moisture content	pH value
PHARM 1	4.6935	4.0225	0.6710	16.68	7.6
PHARM 2	5.5740	4.7000	0.8740	18.59	7.5
PHARM 3	5.5980	4.7190	0.8790	18.62	7.5

Table 2. Total digestion of metals (mg/kg)

Sampling points	Cd	Cr	Ni	Pb	Co	Cu	Zn	Mn	Ca	Fe
PHARM 1	0.163±0.014	0.250±0.008	0.124±0.007	1.682±0.341	0.970±0.006	1.238±0.005	0.888±0.011	1.184±0.013	6.045±0.046	17.770±0.139
PHARM 2	0.113±0.002	1.499±0.041	0.989±0.020	0.921±0.013	0.756±0.021	0.617±0.005	0.675±0.010	4.169±0.059	1.816±0.022	15.100±0.159
PHARM 3	0.117±0.003	1.249±0.037	1.145±0.008	1.183±0.008	0.872±0.014	0.613±0.003	0.561±0.000	4.243±0.062	2.591±0.091	16.600±0.103
TOTAL	0.393	2.998	2.258	3.786	2.598	2.468	2.124	9.596	10.452	49.47
MEAN	0.13	0.99	0.75	1.26	0.86	0.82	0.70	3.19	3.48	16.49

**Table 3. Fractional concentration of cadmium (mg/kg)**

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.001±0.000	0.044±0.001	0.035±0.001	0.000±0.004	0.001±0.000	0.153±0.006	0.232	0.04	0.078	33.621
PHARM 2	0.011±0.000	0.034±0.004	0.034±0.001	0.008±0.004	0.012±0.006	0.160±0.004	0.221	0.04	0.057	25.792
PHARM 3	0.005±0.003	0.030±0.005	0.044±0.006	0.005±0.002	0.004±0.001	0.207±0.000	0.275	0.05	0.069	25.090
TOTAL	ND	0.108	0.113	ND	0.017	0.520	0.728	0.13	0.204	84.503
MEAN	ND	0.04	0.04	ND	0.006	0.17	0.24	0.04	0.07	28.16

Table 4. Fractional concentration of chromium (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.012±0.008	1.391±0.013	1.455±0.007	0.416±0.007	0.630±0.004	0.207±0.014	4.087	0.68	2.834	69.317
PHARM 2	0.025±0.000	0.102±0.017	0.129±0.013	0.786±0.037	0.708±0.010	1.207±0.029	2.957	0.49	0.256	8.657
PHARM 3	0.129±0.014	0.265±0.001	0.210±0.001	0.199±0.000	0.176±0.024	0.437±0.011	1.416	0.24	0.604	42.655
TOTAL	0.142	1.758	1.794	1.401	1.514	1.851	8.460	1.410	3.694	120.629
MEAN	0.04	0.58	0.59	0.46	0.50	0.61	2.82	0.47	1.23	40.20

Table 5. Fractional concentration of nickel (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.034±0.007	1.048±0.011	1.085±0.022	0.267±0.003	0.338±0.009	1.563±0.034	4.335	0.72	2.167	49.988
PHARM 2	0.016±0.003	0.183±0.003	0.182±0.001	0.035±0.002	0.101±0.032	0.735±0.012	1.220	0.20	0.349	28.606
PHARM 3	0.029±0.012	0.221±0.008	0.219±0.005	0.082±0.001	0.081±0.002	0.957±0.016	1.589	0.26	0.469	29.515
TOTAL	0.047	1.452	1.486	0.384	0.520	3.255	7.144	1.18	2.985	108.109
MEAN	0.015	0.48	0.49	0.12	0.17	1.08	2.38	0.39	0.99	36.03

Table 6. Fractional concentration of lead (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.074±0.026	0.440±0.020	0.498±0.012	0.286±0.011	0.273±0.034	0.764±0.007	2.335	0.39	1.012	43.340
PHARM 2	0.018±0.004	0.318±0.013	0.233±0.011	0.105±0.034	0.132±0.019	0.490±0.003	1.296	0.22	0.569	43.904
PHARM 3	0.030±0.009	0.285±0.002	0.352±0.007	0.185±0.014	0.142±0.005	0.394±0.002	1.328	0.22	0.607	45.707
TOTAL	0.062	1.043	1.083	0.576	0.547	1.648	4.959	0.83	2.188	132.951
MEAN	0.02	0.34	0.36	0.19	0.18	0.54	1.65	0.27	0.72	44.32

Table 7. Fractional concentration of cobalt (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.011±0.003	0.171±0.004	0.174±0.005	0.055±0.005	0.052±0.003	0.663±0.010	1.126	0.19	0.356	31.616
PHARM 2	0.011±0.003	0.105±0.004	0.111±0.004	0.004±0.001	0.024±0.020	0.505±0.011	0.760	0.13	0.227	29.868
PHARM 3	0.001±0.004	0.118±0.004	0.145±0.004	0.031±0.004	0.036±0.000	1.141±0.021	1.472	0.25	0.264	17.935
TOTAL	0.023	0.394	0.430	0.090	0.112	2.309	3.358	0.57	0.847	79.419
MEAN	0.007	0.13	0.14	0.03	0.03	0.76	1.11	0.19	0.28	26.47

3.2.6 Copper

The speciation of Cu is as tabulated in Table 8 and also shown pictorially in Fig. 7.

Apart from the highest concentration of Cu associated with the residual, the metal was observed to be highly bound to the exchangeable, and hence contributed to its increased bioavailability. The concentration of the metal in the carbonate was next to that of exchangeable, then organic/sulfide and Fe-Mn oxide followed accordingly. There was indication of anthropogenic contribution.

3.2.7 Zinc

The speciation of Zn in the soil of effluent channels of the Industry under investigation is as presented in Table 9 and Fig. 8.

The metal concentration was lowest in the water soluble, and highest in the residual form. The concentration in the exchangeable form was next to the highest, and with the carbonate, they constituted the bioavailable fraction of the metal in the soil. The organic/sulfide and Fe-Mn oxide forms also had varied concentrations of the metal

associated with them. Hence the observed trend for the metal speciation was F6 > F2 > F3 > F5 > F4 > F1.

3.2.8 Manganese

The speciation of Mn in the effluent channels soil is presented in Table 10 and Fig. 9.

A close look at the speciation of Mn revealed the mobility picture of the metal considering its concentration in the water soluble form. The concentration of the metal was highest in the residual form and was followed by the carbonate. It may be drawn that the mobility of the metal would increase with a decrease in the pH of the soil as well as an increased salt concentration to the soil due to its concentration in the carbonate and the exchangeable forms respectively. The organic/sulfide and the Fe-Mn oxide forms also had a little concentration of the metal in their fractions too.

3.2.9 Calcium

Presented in Table 11 and Fig. 10 is the the speciation of Ca in the effluents channels of Juhel Industry.
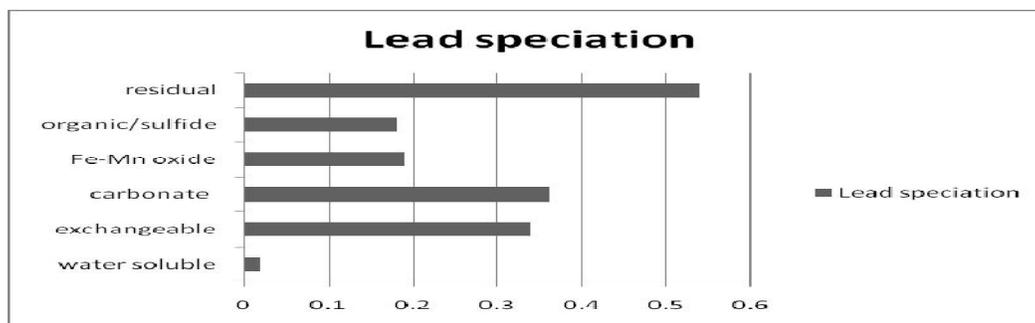

**Fig. 5. Graphical presentation of lead speciation**
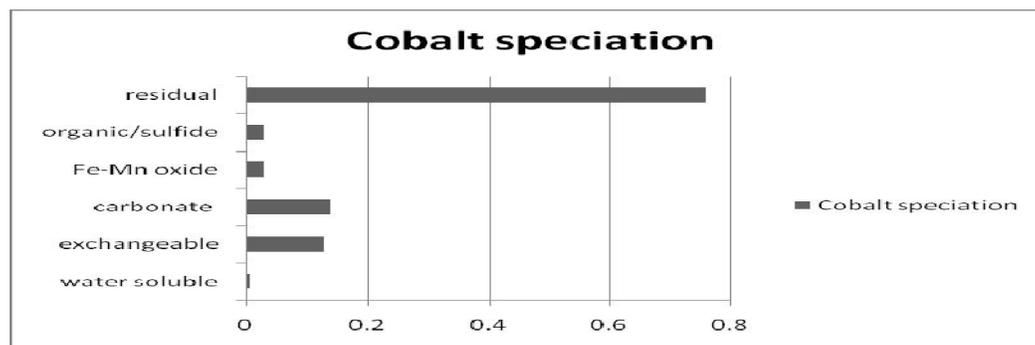
Fig. 6. Graphical presentation of cobalt speciation

Table 8. Fractional concentration of copper (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.014±0.001	1.110±0.016	0.693±0.018	0.399±0.006	0.637±0.000	1.108±0.010	3.933	0.66	1.789	45.487
PHARM 2	0.019±0.001	0.225±0.005	0.256±0.005	0.096±0.001	0.171±0.074	0.304±0.006	1.029	0.17	0.462	44.897
PHARM 3	0.026±0.002	0.195±0.004	0.221±0.006	0.193±0.001	0.163±0.003	0.445±0.002	1.191	0.20	0.390	32.746
TOTAL	ND	1.530	1.17	0.688	0.971	1.857	6.153	1.03	2.641	123.130
MEAN	ND	0.51	0.39	0.22	0.32	0.61	2.05	0.34	0.88	41.04

Table 9. Fractional concentration of zinc (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.139±0.006	0.385±0.005	0.332±0.009	0.240±0.002	0.258±0.004	1.203±0.022	2.557	0.43	0.856	33.476
PHARM 2	0.034±0.002	0.131±0.006	0.108±0.002	0.101±0.001	0.100±0.010	0.864±0.012	1.338	0.22	0.273	20.404
PHARM 3	0.098±0.002	0.076±0.002	0.074±0.003	0.075±0.004	0.067±0.001	0.802±0.024	1.192	0.20	0.248	20.805
TOTAL	0.271	0.592	0.514	0.416	0.425	2.869	5.087	0.85	1.377	74.685
MEAN	0.09	0.19	0.17	0.13	0.14	0.95	1.69	0.28	0.45	24.89

Table 10. Fractional concentration of manganese (mg/kg)

| Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.125±0.009	2.894±0.052	3.015±0.059	2.506±0.067	2.531±0.042	6.095±0.120	17.166	2.86	6.034	33.151
PHARM 2	0.334±0.011	0.882±0.002	0.794±0.039	0.517±0.016	0.491±0.012	2.286±0.032	5.304	0.88	2.010	37.896
PHARM 3	0.108±0.005	1.844±0.059	2.237±0.039	1.817±0.056	1.944±0.037	1.473±0.001	9.423	1.57	4.189	44.455
TOTAL	0.567	5.62	6.046	4.84	4.966	9.854	31.893	5.31	12.233	117.502
MEAN	0.189	1.87	2.01	1.61	1.65	3.28	10.63	1.77	4.07	39.16
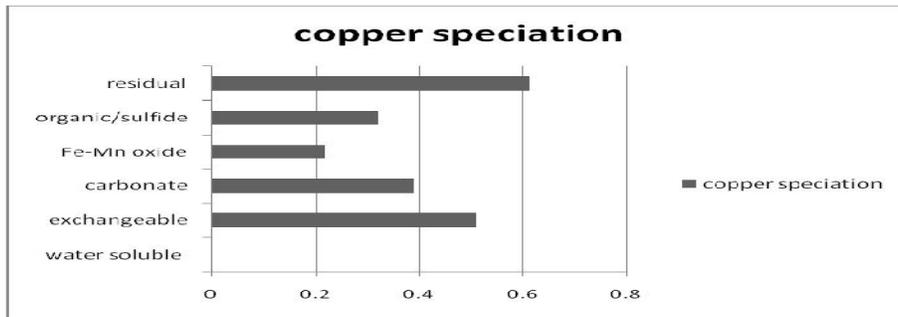
**Fig. 7. Graphical presentation of copper speciation**
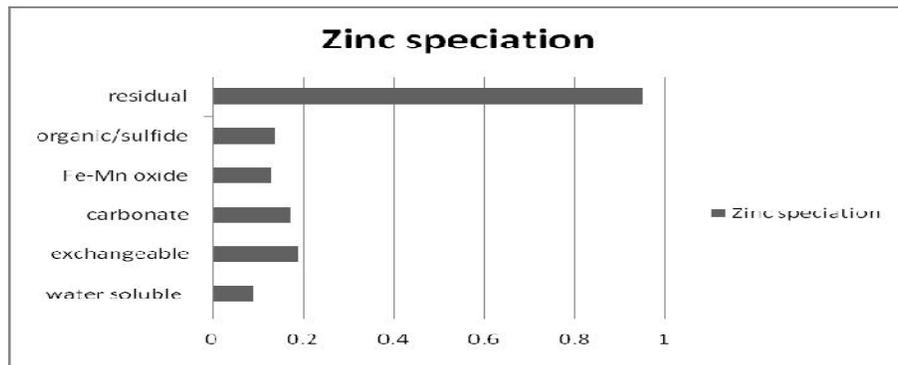
**Fig. 8. Graphical presentation of zinc speciation**
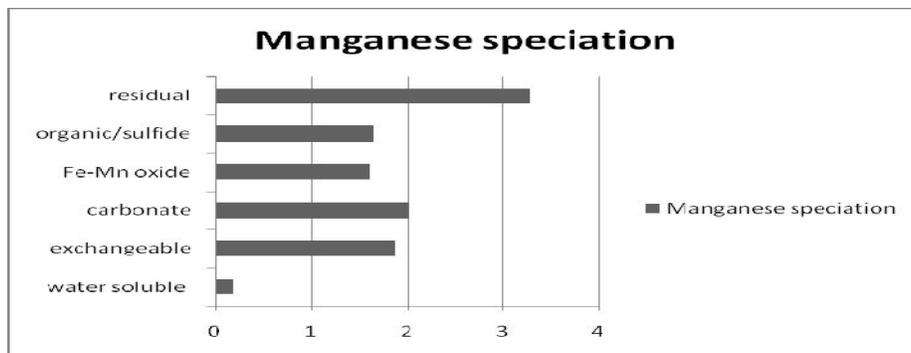

Fig. 9. Graphical presentation of manganese speciation
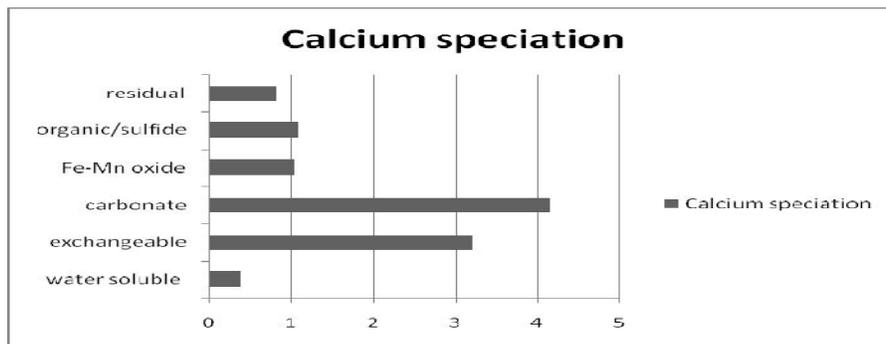
Fig. 10. Graphical presentation of calcium speciation

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Table 11. Fractional concentration of calcium (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.265±0.001	5.041±0.098	5.640±0.093	1.584±0.058	0.791±0.029	0.817±0.020	14.138	2.36	10.946	77.423
PHARM 2	0.511±0.033	2.281±0.065	2.618±0.042	0.128±0.001	0.739±0.024	0.434±0.009	6.711	1.12	5.410	80.614
PHARM 3	0.358±0.044	2.307±0.045	4.213±0.044	1.418±0.068	1.720±0.019	1.235±0.013	11.251	1.88	6.878	61.132
TOTAL	1.134	9.629	12.471	3.130	3.250	2.486	32.100	5.36	23.234	219.169
MEAN	0.37	3.20	4.157	1.04	1.08	0.82	10.7	1.78	7.74	73.05

Table 12. Fractional concentration of iron (mg/kg)

Sampling points	Water soluble fraction (F1)	Exchange-able fraction (F2)	Carbonate bound (F3)	Fe-Mn oxide fraction (F4)	Organic and sulfide (F5)	Residual fraction (F6)	Total	Mean	BAF	%BAF
PHARM 1	0.489±0.104	5.232±0.018	3.811±0.034	3.148±0.043	2.558±0.027	12.910±0.046	27.170	4.53	8.554	31.483
PHARM 2	0.314±0.003	0.543±0.003	0.261±0.004	0.296±0.000	0.252±0.014	14.66±0.258	15.698	2.62	0.490	3.121
PHARM 3	0.719±0.010	2.665±0.076	1.670±0.002	1.134±0.019	1.404±0.004	12.08±0.204	18.234	3.04	3.616	19.831
TOTAL	ND	8.44	5.742	4.578	4.214	39.65	61.102	10.19	12.66	54.435
MEAN	ND	2.81	1.91	1.52	1.40	13.21	20.36	3.39	4.22	18.14

Table 13. Percentage bioavailability of metals

Sample code	Cd	Cr	Ni	Pb	Co	Cu	Zn	Mn	Ca	Fe
PHARM 1	33.621	69.317	49.988	43.340	31.616	45.487	33.476	35.151	77.423	31.483
PHARM 2	25.792	8.657	28.606	43.904	29.868	44.897	20.404	37.896	80.614	3.121
PHARM 3	25.090	42.655	29.515	45.707	17.935	32.746	20.805	44.455	61.132	19.831
TOTAL	84.503	120.629	108.109	132.951	79.419	123.130	74.685	117.502	219.169	54.435
MEAN	28.16	40.20	36.03	44.32	26.47	41.04	24.89	39.16	73.05	18.14

Table 14. Water soluble fractions and the bioavailability fractions of metals

Metals (mg/2g)	Cd	Cr	Ni	Pb	Co	Cu	Zn	Mn	Ca	Fe
Sum of fractions	0.24	2.82	2.38	1.65	1.11	2.05	1.69	10.63	10.70	20.36
Bioavailable fraction	0.07	1.23	0.99	0.72	0.28	0.88	0.45	4.07	7.74	4.22
Water soluble fraction	-0.005	0.047	0.015	0.020	0.007	-0.019	0.090	0.189	0.378	-0.507
%Bioavailability	28.16	40.20	36.03	44.32	26.47	41.04	24.89	39.16	73.05	18.14

The speciation of Ca as shown in the Table typically depicted the behavior of the metal at having a preference for carbonate ion. Its concentration in the carbonate form was highest followed by the exchangeable form. Thus the fate of Ca in the soil of JUHEL, although distributed among other forms, was tangibly bound to the carbonate form as its important phase of chemical association. Anthropogenic contribution was vividly shown by the metal speciation. The observed trend for the metal speciation was F3 > F2 > F5 > F4 > F6 > F1.

3.2.10 Iron

The speciation of Fe is presented in Table 12 and Fig. 11.

Fe was not detected in the water soluble form of the soil, but it had an unusual high concentration in the residual form. That was followed by a very small concentration in the exchangeable form when compared to the concentration in the residual form. Other forms, in which the metal was distributed, had virtually equal levels, except that the carbonate was slightly elevated above the Fe-Mn oxide and the organic/sulfide forms. Hence the observed trend for the metal speciation was F6 > F2 > F3 > F4 > F5 > F1. The bioavailability of the metal was lower compared to that of other metals and with such concentration in the residual, the metal was strongly bound with little or no mobility in terms of solubility in water.

In general, metals partitioning at JUHEL showed that the highest and lowest forms respectively for Fe, Mn, Pb, Cr, Cu, Ni, Co, and Zn are in the residual and water soluble, except for Ca having carbonate bound fraction and water soluble as its highest and lowest forms, as well as Cd having residual and Fe-Mn oxide (reducible) fraction/water soluble as its highest and lowest forms respectively.

Again, in all the metals and the soil considered, an increased bioavailability and hence mobility would be enhanced by remobilization. Remobilization is mainly influenced by four types of chemical changes in soil and water, and they include [7] the following:

- Increased salt concentrations whereby the alkali and alkaline earth cations can compete with the metal ions adsorbed onto solid particle. This is more obtainable for the exchangeable fractions.
- Decrease in the pH, which leads to dissolution of carbonates and hydroxides, and increased adsorption of metal cations due to competition with hydrogen ions (H^+). This is more obtainable in the carbonate forms.
- Changes in the redox conditions, usually in conjunction with a decrease in oxygen potential due to advanced eutropolication iron and manganese hydroxides are partly or completely dissolved, whereby part of the incorporated or adsorbed heavy metal load is being released. This is observed for the reducible fraction.
- Increased use of natural and synthetic complexing agents, which can form soluble complexes sometimes of high stability with heavy metals that are otherwise adsorbed to solid particles.

In addition to these four processes, there are other biochemical transformation processes by means of which the heavy metals are either transferred from sediments to animals or plant organisms.

3.3 Percentage Bioavailability

Having understood that the use of sequential extractions, although more time consuming, furnishes detailed information about the origin, mode of occurrence, biological and
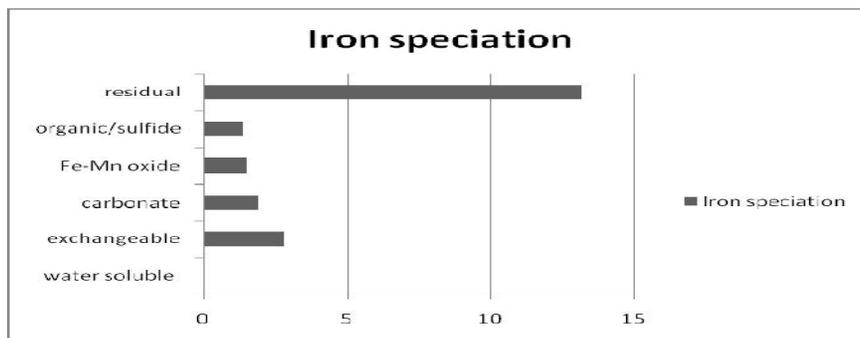

Fig. 11. Graphical presentation of iron speciation

physicochemical availability, mobilization and transport of trace metals, consideration of the percentage bioavailability became necessary to have an adequate picture for adequate concern about the metals content of the soils considered.

Bioavailability could mean the ready availability of elements for plant uptake, in which there exists potential effect to animals including man through the food chain. And recalling that the limiting step for elemental entry to the food chain, as discussed in the factors influencing bioavailability in soil, usually occur at the soil to the root; and this critical step usually depends on element concentrations in the soil pore solutions, which are controlled by local soil physical and chemical conditions including water content, pH and other factors, the percentage bioavailability was computed from the water soluble, exchangeable and carbonate fractions and presented in Tables 13,14.

4. CONCLUSION

The percentage moisture content was relatively low (<20%) and the pH was slightly neutral (7.5 – 7.6). The total metal concentration of Fe was found to occur at elevated concentration in all the three samples. Calcium was seen to be the most bioavailable followed by Pb while the least bioavailable metal was Fe. The levels of incidence of the considered metals in the soils studied are insignificant to conclude pollution of the environments.

There was significant level of anthropogenic contributions in the environments judging with the speciation results; but the risks are still below the tolerable range considering the standards.

Iron, (Fe) being very significant in terms of pollution or concentration in the soil was the least bioavailable; secondly Fe is an essential element and therefore does not really connote the hazards sought for the environment, with respect to the presence of these industries. Metals speciation of the soil samples showed that the highest and lowest partitions were found in the residual and water soluble for the metals Fe, Mn, Pb, Cr, Cu, Ni, Co and Zn, respectively except

for Ca which had its highest concentration in the carbonate bound partition, and the lowest concentration in the water soluble fraction. The highest fraction for Cd was found in the residual and Fe-Mn oxide (reducible) forms, with the water soluble fraction having the lowest Cd concentration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nilanjana D, Vimala R, Karthika P. Biosorption of heavy metals- An overview, Indian Journal of Biotechnology. 2008;7: 159-169.
2. Farmaki EG, Thomaidis NS. Current status of the metal pollution of the environment of Greece- A review. Global Nest Journal. 2008;10(3):366-375.
3. Hart BT, Lake PS. Studies of heavy metal pollution in Australia with particular emphasis on aquatic systems, In: Lead, Mercury and Arsenic in the Environment, Editors: Hutchinson TC, Meema KM, John Wiley and Sons Ltd. 1987;187-190.
4. Nigeria: Mass lead poisoning from mining activities, Zamfara State - Update 1. Available:http://www.who.int/csr/don/2011_11_11/en/index.html (Assessed 11.11. 2011)
5. Krishna AK, Govil PK. Soil contamination due to heavy metals from an industrial area of Surat, Gujarat, Western India. Environmental Monitoring and Assessment. 2007;124(1-3):263-275.
6. Tessier A, Campbell PGC, Blsson M. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry. 1979;52(1):45–53.
7. Omuku PE. Speciation patterns of selected Heavy metals in the sediments of roadside gutters in Awka metropolis, Anambra State, M.Sc Project, Department of pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka. 2008;9-31.

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