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Fractional Characterisation of Bioavailable Arsenic in Poultry-Litter Amended Soil by Geochemical Mobility Analysis and XRD Technique

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ABSTRACT

Many farmers are using poultry litter as an organic fertilizer. Unfortunately, a significant portion of this litter comes from commercial broiler operations that use feeds containing arsenic (As) as an additive to control parasites and increase poultry weight. Most of the ingested arsenic does not accumulate in the poultry meat but is excreted by the poultry. As a result, nearly 90% of arsenic feed to poultry ends up in their waste. The presence of As in the poultry litter used as fertilizer potentially accumulated in soils and cause soil contamination. In this study, five steps of Tessier method were adopted for the geochemical partitioning of As to identify the fractional forms that are liable to leach from the soil matrix amended with poultry litter. Also, X-ray diffraction (XRD) was used to determine As association with soil components among the fractional forms. The results observed indicated higher fractions of arsenic in the exchangeable phase for all the samples when compared to the residual. This suggests As can be released or desorbed as it was not tightly bound to the soil matrix and thus, could be a source of soil contamination. Furthermore, the XRD results indicated that the bulk chemistry of the soil samples is dominated by quartz followed by aluminosilicates (comprise of orthoclase, muscovite, and clinochlore) all of which could be the sorption sites or sinks for As. However, As can be released from such sorption sites by desorption and dissolution of Fe oxides in response to changes in physicochemical conditions of the soil environment and thus, possibly influence the As mobility. Accordingly, the use of poultry litter as a fertilizer and source of organic matter needs to be monitored and regulated periodically, to safeguard soil health quality and by extension prevent total environmental pollution through dispersion.

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1. Introduction

The ever-growing population in the world results in an increased demand for food supply. This has necessitated intensive farming, which resulted in a decrease in soil fertility. To solve the problem of soil fertility decrease, farmers

long ago have resorted to be using both inorganic and organic fertilizers to augment soil fertility. Organic fertilizers are mineral sources that are naturally available and contain a moderate amount of plant essential nutrients. They are capable of mitigating the issues caused by synthetic fertilizers. They reduce the need for synthetic fertilizer applications to maintain soil fertility. Amongst organic fertilizers, poultry litter has recently gained great interest. They gradually release nutrients into the soil solution while maintaining nutrient balance for crop plant

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growth. However, most of the arsenic used as an antibiotic in commercial broiler production ends up in the litter and therefore, using this litter as a soil amendment can cause serious soil environmental problems. This is because many organic farmers use fresh, composted and/or pelleted poultry litter as a fertilizer and source of organic matter. A significant portion of this litter comes from commercial broiler operations that use arsenic as a feed additive to control parasites and increase weight. Most of this arsenic does not accumulate in the poultry meat but is excreted by the birds. As a result, nearly 90% of arsenic fed to poultry ends up in their litter. Because this heavy metal has the potential to accumulate in the soil and thus, can cause health issues related to soil and water contamination.

Organic manure remains the main nutrient source that substitutes chemical fertilizers used in agricultural activities (Kabir et al., 2019). As an additive in feeds, As is confirmed to be used in the form of drugs (roxarsone) as a supplement to prevent disease and improve feed efficacy and weight gain (Ogunwale et al., 2022). However, it largely excreted in organic form in poultry litter (Morrison et al., 1969), which might be converted to inorganic such as arsenate (+5) and arsenite (+3) under microbial activities and photolysis (Fisher et al., 2011). In due course, a substantial amount of As is introduced into the environment due to the frequent and continuous disposal of poultry litter (Garbarino et al., 2003). A higher concentration of As was confirmed in the soil amended with poultry litter containing roxarsone (O'Connor et al., 2015). The widespread use of arsenic feed as additives by commercial poultry feed producers, complemented by improper disposal of poultry waste, has long-standing implications of creating unnecessary risks of water and soil ecosystems contamination near chicken farms.

Arsenic is a toxic metalloid that enters the soil and water environment naturally or anthropogenically in both organic and inorganic compounds with variable oxidation states. The common oxidation states are arsenate (+5), arsenite (+3), and arsenic (Mandal 2002; Jang et al., 2016). Furthermore, the partitioning of arsenic onto soil depends on its oxidation state because of its oxyanionic characteristics. The retention of arsenate to mineral constituents of soils such as clay, oxides, and organic matter is higher compared to arsenite that is more complicated and dependent on specific soil chemical conditions, size, and charge (Pongratz, 1998; Nachman et al., 2008). Metal oxides stabilize arsenic, while organic matter allows solubility to occur as a result of their negative charge. However, an investigation revealed strong sorption of such metal oxides as amorphous $Al(OH)_3$, hydrous ferric oxides (HFO), and hematite. On the other hand, arsenite was found to have adsorption affinity on goethite, ferrihydrite, and kaolinite (Arai, 2002). Also, it was confirmed that retention of As even occurs at natural soil pH (Pantsar-Kallio, 1997).

Because the soil matrix is heterogeneous, attention must be paid to the interaction of different forms of arsenic (As) with different soil constituents. This is because an accurate description of the complex interactions of As forms in soils are required for predicting their behaviour in the soil environment. The dispersion of As contaminants from the poultry-litteramended soil into the surrounding environment is determined by their release and transport characteristics. As a result, the characterisation of As in soil should go beyond determining total content. This is because total metal concentrations provide little or no information on the potential mobility and bioavailability of toxic metals. The geochemical phases of toxic metals will provide more information about metal transfer along the soil-water-plant-animal-human chain (Rattan et al., 2005). In addition, since the chemical variability of arsenic stems from its electronic structure and bonding properties, which give rise to a variety of forms in the solid, aqueous, and gas states and therefore, understanding the mineralogy of the

substrate (soil) and the fate of As on the surface charge of the substrate during adsorption will aid in the long-term storage and management of the substrates after adsorption. In this context, a mineralogical method could be used to estimate the geochemical status and potential mobility of As. Direct or indirect determination of solid metal phases is possible. The indirect method involves the sequential extraction of fractions of a metal bound to the soil, whereas the direct method employs spectroscopic instruments to identify minerals (Selim and Park, 2001).

The sequential extraction of metals in soil is the more commonly used of the two techniques. However, the main issue with extraction techniques is that each extractant affects the soil components differently, resulting in artifacts. Furthermore, the method is heavily influenced by variables such as extractant selection and order, process length, solid/liquid ratio, and sample preparation and conservation procedures (Filgueiras et al., 2002). Because of these issues, several authors have advocated for the use of direct techniques to determine how toxic metals are associated with various soil components (Beesley and Marmiroli, 2011) and thus complete the information on the solid phases that directly retain toxic metals. For detecting and identifying toxic metals in different geochemical soil phases, different direct characterisation methods were chosen, such as Field Emission Scanning Electron Microscopy (FE-SEM) with
Energy Dispersive X-ray Spectroscopy (EDX), X-ray diffraction (XRD), and Time of Flight Secondary Ion Mass Spectrometry (TOFSIMS) (Arenas-Lago et al., 2015; Kierczak et al., 2008). Even though the techniques are semi-quantitative, they provide detailed information about the nature of the soil components and potential interactions with toxic metals. For example, Gee et al. (1997) investigated the mineralogy of smelting slags as well as the response of specific mineral forms to natural weathering processes and potential release into the environment. Similarly, the goal of this study was to investigate and predict the mobility and dispersion of As from poultry-litter amended soil into nearby aquatic and terrestrial environmental compartments using both indirect and direct techniques. This is to reduce the shortcomings of indirect technique, as direct approach of using sophisticated XRD tool (as a nondestructive technique for characterizing crystalline materials) provides direct identification of phases, crystal structure, lattice parameters, chemical composition, structural information, about crystalline materials (Yuji et al., 2005). Therefore, this study uses both direct and indirect approaches to address the challenges of using only one approach. The objective was to determine mobility and bioavailable fractions of arsenic in poultry-litter amended soil. Because information on geochemical phases is crucial as it explains trace metal fraction available and liable to be absorbed by the plant and/or animal to cause adverse effects on humans or other organisms (Rattan et al., 2005). In addition, accurate determination of the specie form of As in natural soil is therefore fundamental to predicting its transport in the environment for environmental regulation. Hence, this was the focus of this study.

2. Materials and Method

2.1 Soil samples collection

Soil samples (0-40cm) were collected from an uncontaminated site with no history of poultry litter application using a core sampler as described by Xie et al., 2015.

2.2 Poultry Feed and chicken growth experiment

Agro tech broilers (day old) were purchased from vendors, divided into two (2), and fed with a series of different feeds comprised of starter feed for 32 days, followed by finisher for the remaining 12 days (Anosike et al., 2020). The poultry was grown in an environmentally controlled storehouse. In the storehouse, age-

related temperature and ventilation protocols were followed as per the management guides of poultry farming.

2.3 Poultry litter sampling and Analysis

Poultry litter with debris was collected at the end of each diet feed (starter and finisher) to allow maximum accumulation, the litter was collected at 32 and 44 days and composted for 45 days under moisture content of 50-60 %, turned at intervals to maintain porosity. A portion of each sample was air dried, ground, and passed through a 2mm sieve for use in the laboratory for determination of total As concentration. pH was determined using a digital pH meter, total organic carbon (TOC) using Walkley and Black procedure. The cation exchange capacity (CEC) using neutral KCl saturation and neutral CH_3COONH_4 displacement, while particle size tests by hydrometer procedure.

2.4 Soil amendment incubation experiment

Four composted poultry litter application rates (10% dry weight) were amended with soil samples under aerobic treatments and were placed in plastic vessels open to the air and turned over every three (3) days. The incubation experiment lasted for 90 days, moisture of the whole treatment was maintained at 30% by periodically weighing these vessels and compensating for any weight loss throughout the experiment (Xie et al., 2015).

2.5 Digestion of Soil and Poultry Litter for determination of Arsenic

The amended poultry-litter soil samples were weighed 0.5g into a 125 ml Erlenmeyer flask and digested with a mixture of conc. HNO_3 and conc. H_2SO_4 (32.5:4:1 v/v) under a fume hood. The mixture was then cooled and filtered into a 100ml volumetric flask and the volume was made up to the mark with distilled water. The concentration of arsenic was then determined using Atomic Absorption Spectrophotometer (AAS). All soil samples were immediately lyophilized, ground, and divided into portions for fractionation using the Tessier method and XRD characterisation of thin film samples in ARL'XTRA X-ray.

2.5 Fractionation Procedure

The Tessier method was chosen because it is the most effective, acceptable and widely used approach for the geochemical analysis of soil, sediment, sewage sludge and dust. The method compared findings obtained in the five steps process and confirm the fair bonding and chemical partitioning of heavy metals in five phases such as exchangeable metals, associated with carbonates, associated with Fe–Mn oxides, associated with sulfides and organic matter, and residual fraction. The fractionating procedures are described as follows:

Step 1- Exchangeable Fraction

1g of soil was placed into a 100ml beaker, added 20ml of 1 M $MgCl_2$ and stirred for 1hour at ambient temperature. The solution was centrifuged at 4500 rpm for 15min and then decants the supernatant using Whatman No. 42 filter paper in a conical flask.

Step 2 - Associated with Carbonates

From the residual soil of step 1, added 20ml of 1M $NaC_2H_3O_2$ (sodium acetate/acetic acid buffer) stirred. The solution was centrifuged at 4500 rpm for 15min and then decanted the supernatant using Whatman No. 42 filter paper in a conical flask.

Step 3 - Associated with Oxides

From the residual soil of step 2, 20 ml of 0.4 M NH_2OH (sodium acetate/acetic acid buffer) was added. Shake at 96 ^{0}C in a water bath for 6 hours. The solution was centrifuged at 4500 rpm for 15min and then decanted the supernatant using Whatman No. 42 filter paper in a conical flask.

Step 4 - Associated with Organics

The residue from step 3 was oxidized in which 5ml each of 0.02 M HNO_3 and 30% H_2O_2 was added and heat the mixture for 2 hours with intermediate shaking and allowed to cool. Again, added another 3 ml of 30% H_2O_2. Heats the mixture at 85 °C again for 3 hours with intermittent shaking and allowed it to cool. Again, 5ml of 3.2 M ammonium acetate ($NH_4CH_4CO_2$) then dilute to a final volume of 20 ml. The solution was centrifuged at 4500 rpm for 15 min and then decanted the supernatant using Whatman No. 42 filter paper in a conical flask.

Step 5 - Residual

The residue from step 4 was oven dried at 105 °C. Adds 25 ml of the mixture of 5 ml (70%) HNO_3 (40%), 10 ml hydrofluoric acid and 10 ml (60%) of perchloric acid ($HClO_4$) in a beaker. The solution was centrifuged at 4500 rpm for 15min and then decanted the supernatant using Whatman No. 42 filter paper in a conical flask.

2.6 Soil Mineralogical Analysis

Soil mineralogical analysis was performed in an X-ray diffractometer (Thermos scientific model: ARL'XTRA X-ray). The instrument was equipped with a graphite-diffracted beam monochromator and copper radiation source [λ (Kα1) = 0.15406 nm], operating at 40 kV and 30 mA. The X-ray powder diffraction pattern (XRPD) was collected by measuring the scintillation response to Cu Kα radiation versus the 2θ value over a 2θ range of 2–70°. The identification and quantification of the crystalline phases were performed using an installed software program (Crystal Impact). These extremely acidic conditions favor the mobility and availability of heavy metals in the soil.

3. Results and Discussion

3.1 Soil physicochemical characteristics

The results of the physicochemical parameters of soil samples amended with poultry litter are presented in Table 1. The parameters determined were soil pH, organic carbon, CEC, clay, silt, and sand contents. The soil pH was neutral, organic carbon was within the recommended range, CEC was moderate, while the soil was largely dominated by silt content. The neutral soil pH was within the agricultural limit of 6 – 8. However, an increase in pH declines heavy metals' mobility (Ogunwale et al., 2021). The organic carbon of agricultural soil was 3.44% and within a range between 2.8 – 4.8% for 30 -100cm depth. Consequently, could support the retention of metals at the soil surface. While CEC is 6.84 Cmol(±)/kg which is moderate in silt-clay soil, which is perhaps attributed to a high degree of surface negative charges and adsorption. Meanwhile, the clay content was 21% indicating a high percentage of silt. This result supports the availability of micronutrients and heavy metals, which could support their sorption for plant growth. On the other hand, the observed result could influence heavy metals' bioavailability, mobility, and precipitation of hydroxides, carbonates, or insoluble organic substances in soil.

Table 1. Physicochemical characteristics of poultrylitter amended soil.

Parameter	*Measurements*
pH	7.1
CEC (Cmol(±)/kg	6.68
Organic Carbon (%)	3.44
Clay Content (%)	21
Silt Content (%)	75
Sand Content (%)	4

3.2 Total arsenic concentration in Poultry litter amendment soil and Non-poultry litter amended soil

The results of total As content in the poultry-litter amended soil and non-poultry-litter amended soil are presented in Table 2. The total concentration of As in non-poultry-litter amended soil was observed to be 23.65 mg/kg, while the poultry-litter amended soils that comprised of Chikun starter (Chikun A), ultimate starter (Ultimate A), Chikun finisher (Chikum B) and ultimate finisher (Ultimate B) were observed to be 75.3 mg/kg, 33.8 mg/kg, 27.0 mg/kg, and 23.1 mg/kg, respectively. The As concentration distribution pattern was found to be in the following sequence: chikun A > chikun B > ultimate A > ultimate B > non-poultry-litter amended soil. This implies that all the poultry-litter-amended soils had the highest content of As relative to non-poultry-litter amended soil. Consequently, the highest As content found in the poultry-litter amended soil was largely necessitated by the intrusion of the metal from the poultry feed used in the study. The highest concentration of As in both chikun A and B litter may be attributed to cumulative feed consumption of the starter feed used within the 35 days length of feeding time. This could further be supported by the fact that as the poultry grew over time, their rate of consumption progressively increased and thus, more content of As was taken up by the poultry. However, the low content of As observed in chikun finisher (CF) and ultimate starter (US) may be associated with the replacement of grower feed by the finisher feed or diet, which was mainly used for 14 days. Besides, the rate of consumption was perhaps reduced after the 35 days of growth and therefore, declined the uptake of As from the finisher feed. Overall, applying poultry litter for soil fertility amendment can necessitate soil contamination with a high concentration of As, which can substantially add an intolerable concentration of arsenic beyond the carrying capacity of the soil environment through continuous accumulations.

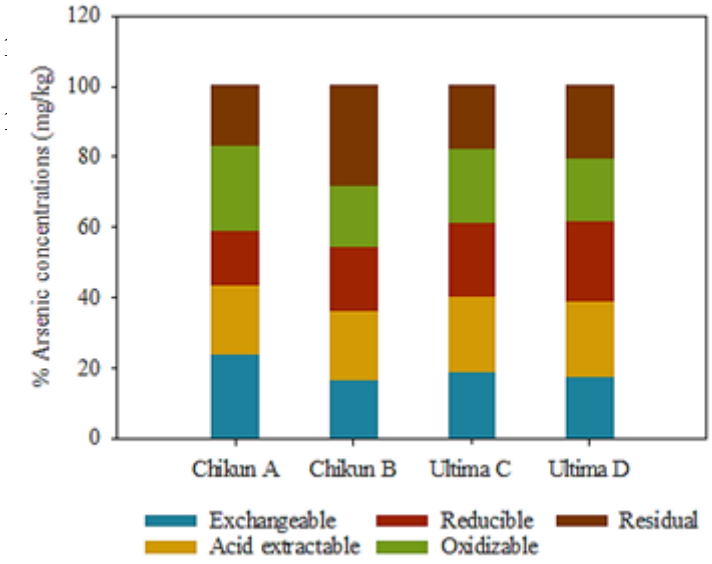
Table 2: Arsenic Concentration soils (Poultry litter amendment and Non-poultry litter amended Soil).

Samples	Arsenic Concentration (mg/kg)
Chikun A	75.30
Chikun B	33.80
Ultima C	27.06
Ultima D	23.10
Non-amended poultry litter soil	21.65
EPA Standard	40.00

3.3 Chemical fractionation of As in soils amended with poultry litter

The result of the five steps of sequential extraction of arsenic is presented in Fig. 1. The percentage of the fractions observed indicated that for chikun A amended soil exchangeable had the highest percentage (23.42%) and the lowest percentage observed was reducible with 15.76%. The residual fraction observed in that soil was 17.22%. Furthermore, the fractions observed for chikun B indicate 28.1% to be residual with the highest fraction while exchangeable was 16.0% with the lowest fraction. The fractions for both ultima A and

ultima B were similar with 18.4% and 17.31% observed as exchangeable. The overall distribution profile of As in these samples was in the following sequence: acid extractable > oxidizable > reducible > exchangeable > residual. However, exchangeable and acid extractable were observed to have the highest fractions in all the samples. Exchangeable arsenic can be easily replaced or lost because it is not tightly bound to the soil. The percentage of exchangeable and acid extractable relative to the total amount could be seen as a mobility factor (MF) and therefore, the mobility of As from the poultry-litter amended soil was easily possible. On the other hands, As attached to reducible fractions in chikun A and ultima A was more tightly bound to the soil. However, MF combines exchangeable, acid extractable, and reducible considering their mobility potential. On the contrary, the sum of the fractions is oxidizable and residual are comparatively stable and difficult to lose in a short time. Oxides occurred as bound substances between soil particles, thereby could be mobilised under reducing and acidic environments while the organic phase is under natural phenomena but can be mobilised under high oxidizing environments usually caused by decomposition of organic matter (Zimmerman and Weindorf, 2010). This indicates that arsenic in the Chikun A and B amended soil was not bound to the soil. The significant presence of As in reducible (bound to Fe/Mn oxides) and other phases except for residue of all the poultry-litter amended soil might be attributed to the high stability constant of As (Shrivastava and Banerjee, 2004; Garcia-Manyes et al., 2002).



Acid extractable Oxidizable

Figure 1. Chemical fractionation of As in soils amended with poultry-litter

3.4 Bioavailable, non-bioavailable, and total As in soils amended with poultry-litter

The results for the bioavailable, non-bioavailable, and total as in soils amended with poultry litter are presented in Fig. 2. The result indicates that the percentages of the bioavailable fractions for the amended soils were 23.42% and 16.28%, which are the highest and the lowest observed in chikun A and B amended soils, respectively. The level of As contamination in soil is measured by its extent of bioavailability.

The severity of As contamination in soil is regulated by the total As content and bioavailability. The bioavailable fraction of As in soil is considered to be a detrimental measure in soils affecting plants and animals and subsequently humans (Singh et al., 2020). The water soluble and exchangeable fractions

are considered to be mobile and bioavailable (Balasoiu et al., 2001; Tessier et al., 1979).

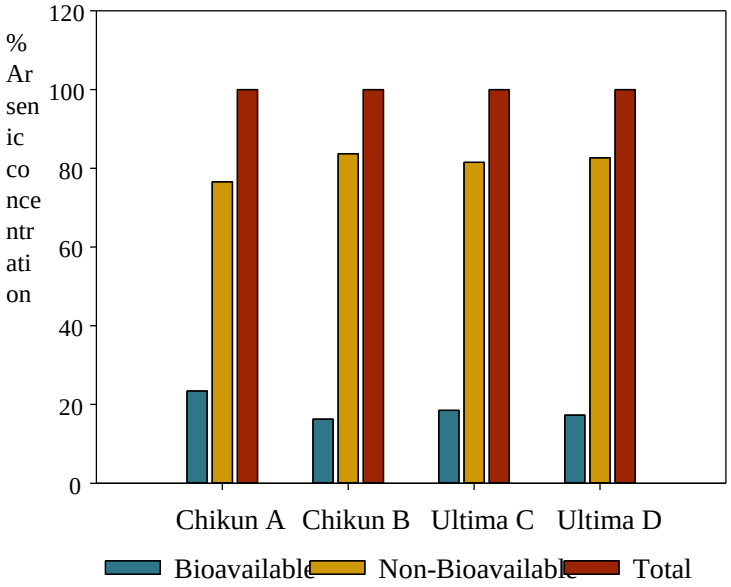


Figure 2. Bioavailable, Non-bioavailable, and Total As in soils amended with poultry-litter

3.5 Mineralogical analysis of poultry-litter amended soils and Non-amended poultry-litter soil by XRD technique

The results of mineralogical analysis of soil components bound to arsenic in poultry litter amended soils along with soil samples free of poultry-litter amendment by XRD technique are presented in Figure 1-5. The investigation revealed a dominance of quartz SiO_2 (68-48%), followed by orthoclase (24-19%), and then albite (24-19%), clinochlore (16-3.22%), and finally osumilite (3.0-0.5%). In the dominance hierarchy, quartz was at the top, followed by orthoclase, muscovite, albite, osumilite, and then clinochlore, as presented in Fig 1-4. Therefore, the As content in poultry litter used to amend the non-amended poultry-litter soilwas largely associated with quartz and orthoclase. X-ray diffraction (XRD) is a technique that can provide detailed information onminerals**,** crystallinity, and structure of solid samples. The minerals can be a major component of a solid phase crystalline and/or amorphous, a surface associated component, or a trace component of the bulk phase (Bertsch et al.,1998). Because As is characteristically present in the poultry-litter amended soil, its surface adsorption and desorption behavior on soil mineral surfaces can play a vital role in regulating its levels in soil solution. In this context, since As has a strong sorption affinity for silicate and aluminum oxides or oxyhydroxide minerals, surface sorption or coating on these mineral surfaces are thought to be very important sinks or sorbents of As content in the soil environment (Manceau et al., 2007). Other mineral sinks or reservoirs, such as clays and manganese oxides, may absorb arsenic as well, especially in low-iron environments. Hence, in this case, As can be sorbed by quartz in a strong bind mechanism. Arai and Sparks (2002) reported that As has been found to increase with increasing pH when attached to minerals. The surfaces of quartz and other minerals are coated with nanometer-size iron (Fe) and aluminum (Al) oxides and/or silicates, which control the adsorption properties of arsenic and can be released by desorption and dissolution of Fe oxides in response to changing chemical conditions. This implies that even when As is sorbed in the above-mentioned minerals, it can be set free and become mobile and eventually bioavailable to the plant for uptake and consequently causes adverse effects on the human and the environment.

Although the pH of the soil sample was neutral, with a change in the pH condition of the soil environment, As can be significantly released into the proximate aquatic and terrestrial ecosystem and cause ecological imbalances. Therefore, soil fertility amendment with poultry litter could potentially be a source of ecological contamination. Even though the XRD revealed that the mineralogy is dominated by quartz and aluminosilicates, which can strongly bind to As affecting its mobility but with a decrease in pH can leach into the environment and become a source of contamination. Hence, soil fertility amendment with poultry litter requires monitoring and regulation.

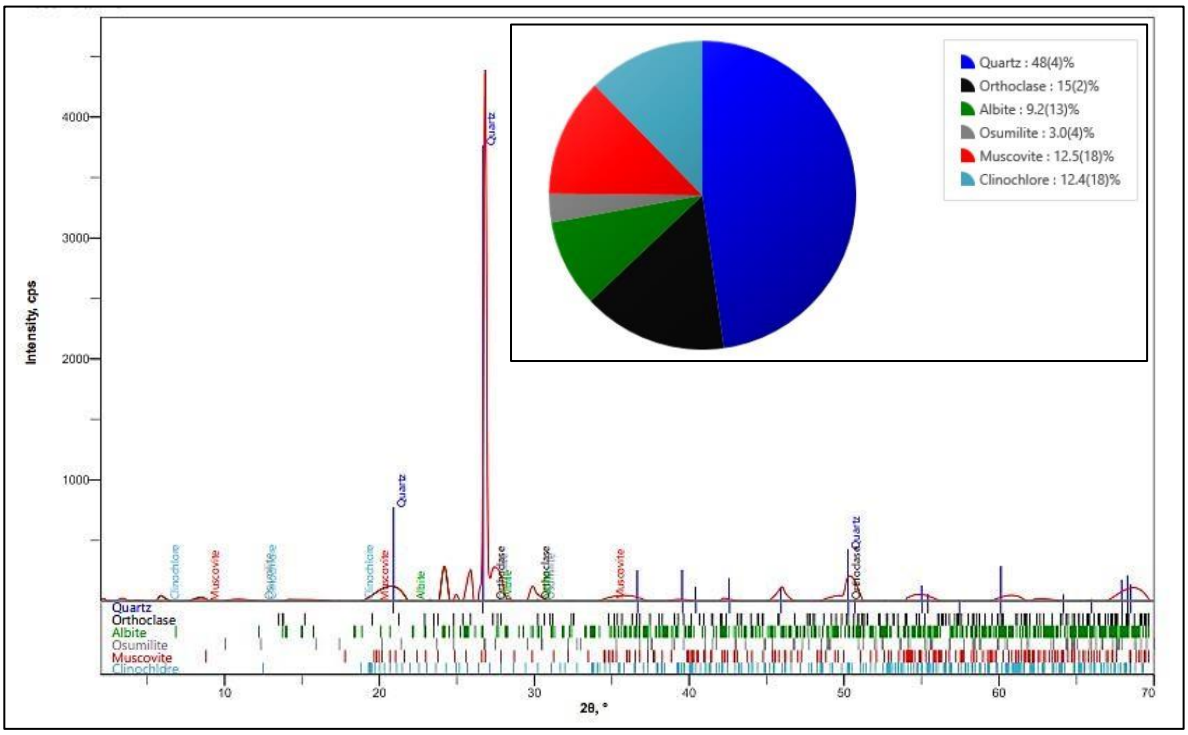


Figure 1. Mineral content in CS-litter amended soil.

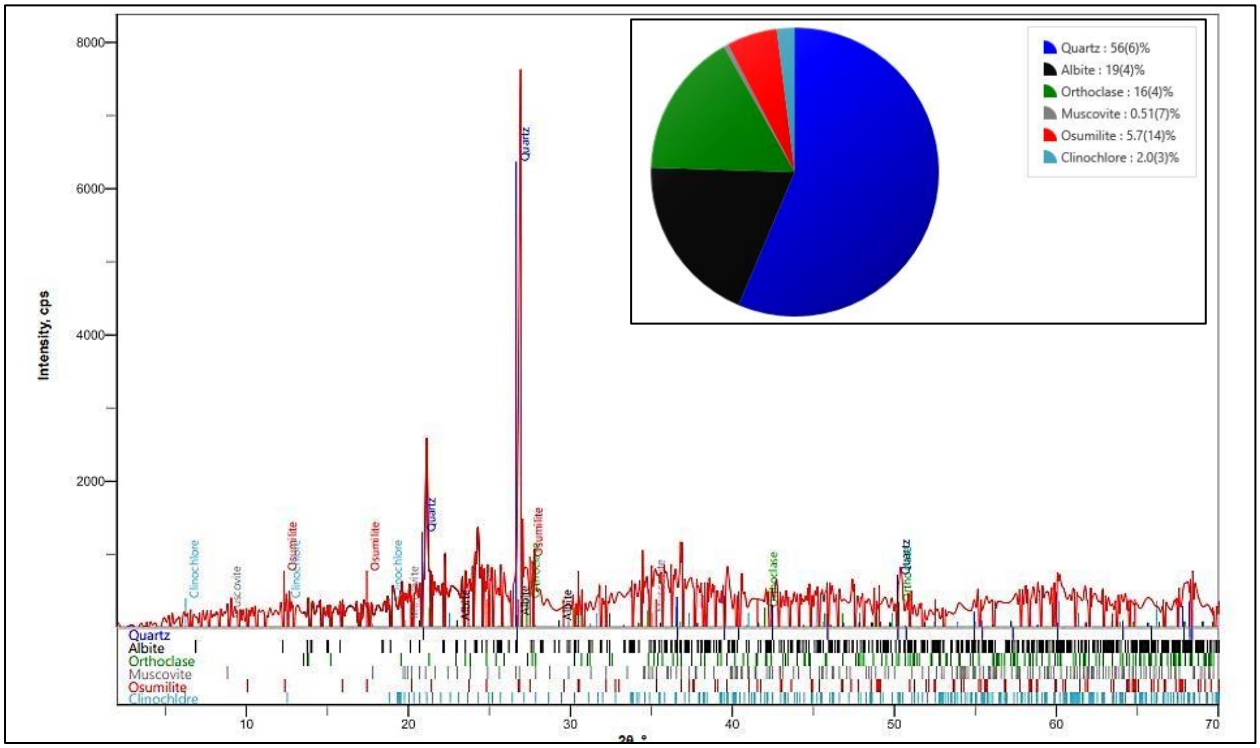


Figure 2. Mineral content in US-litter amended soil.

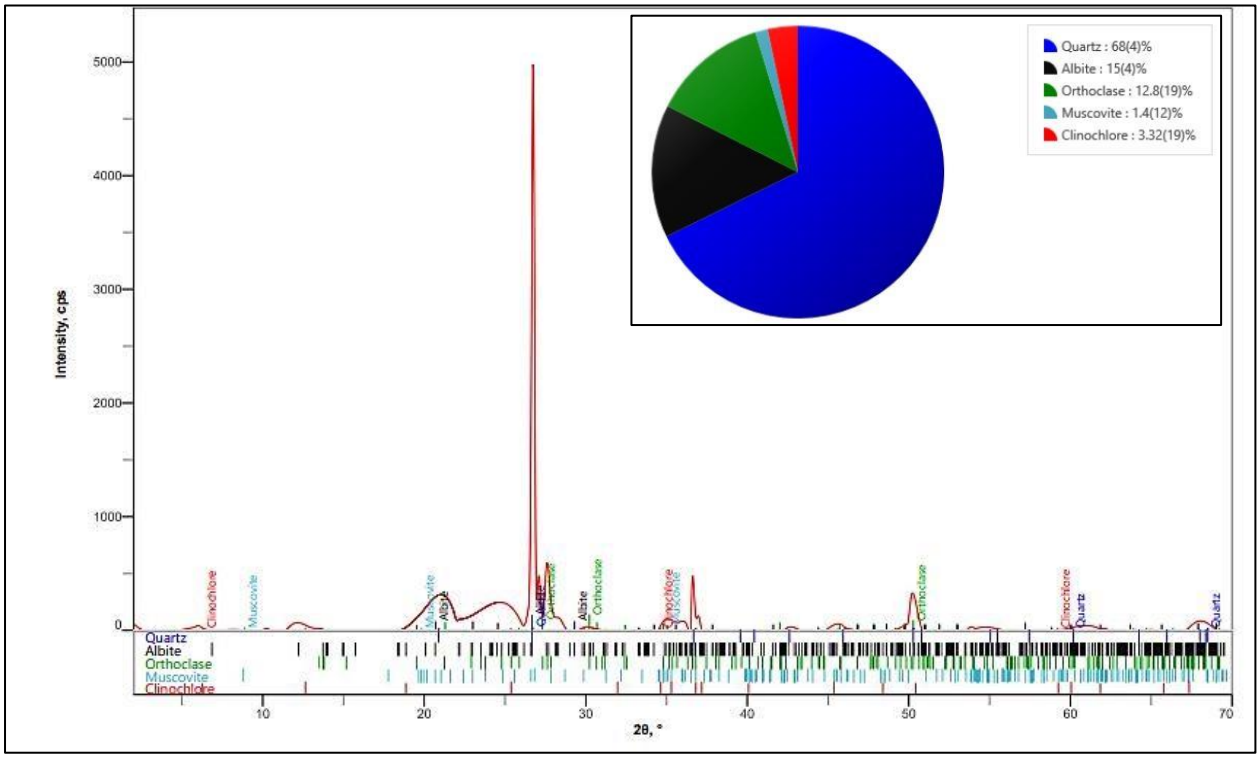


Figure 3. Mineral content in CF-litter amended soil.

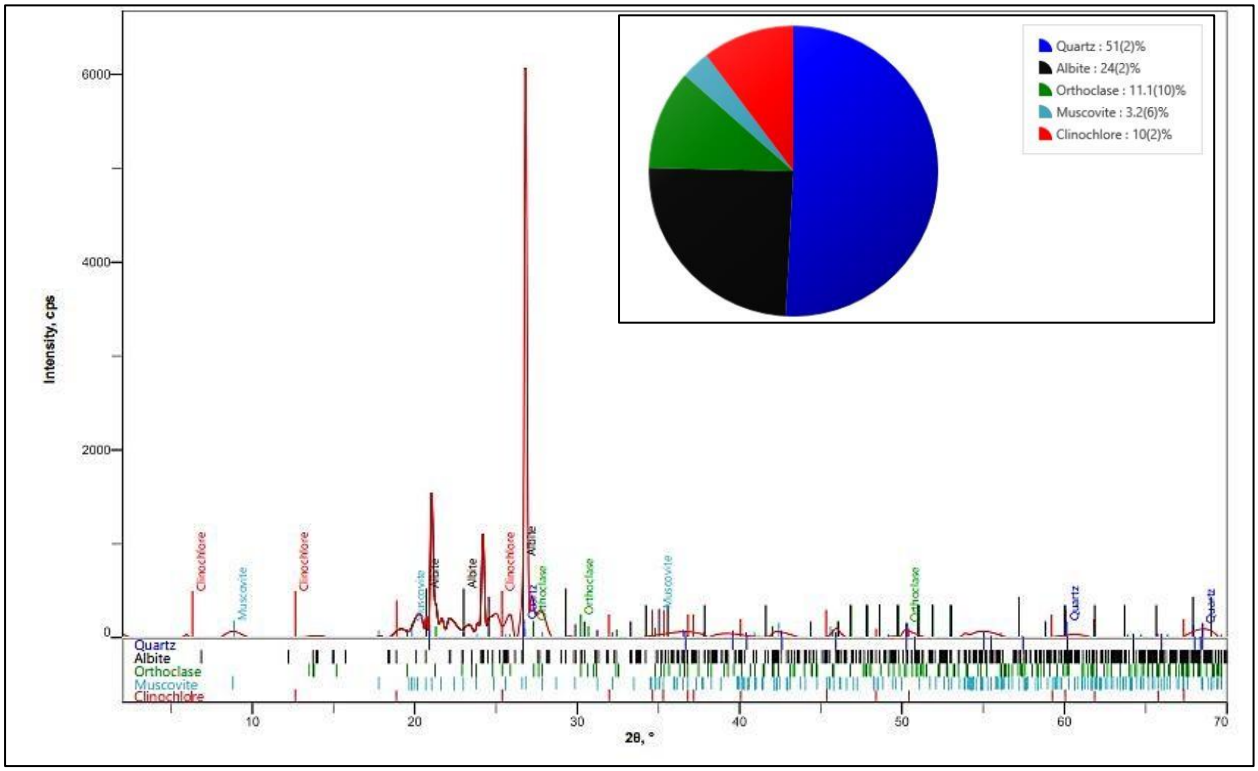


Figure 4. Mineral content in UF-litter amended soil.

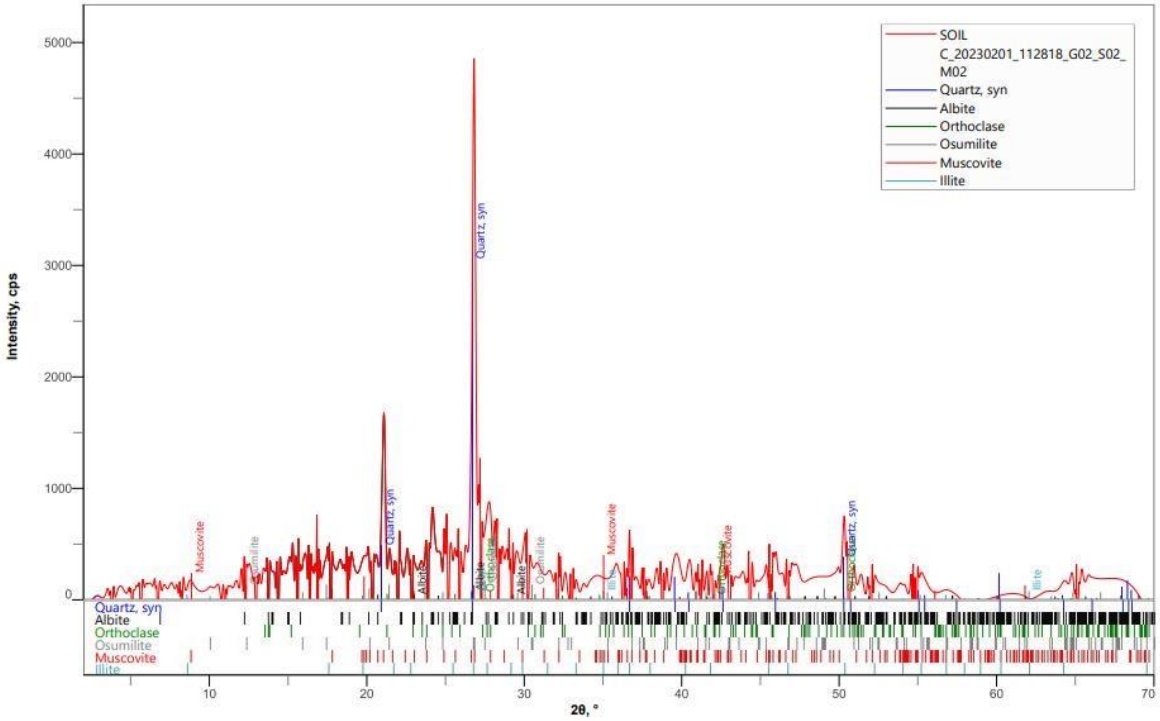


Figure 5. Mineral content in soil free of poultry-litter amendment.

Conclusion

The findings of this research work revealed that poultry-litter amended soils contained high concentrations of As. From the side of fractionation, results revealed that poultry-litter amended soils contained As in different fractions but with dominance in the bioavailable phase. On the other hand, according to the mineralogical findings, the soil minerals were primarily orthoclase, albite, clinochlore, and finally osumilite containing significant amounts of quartz. Accordingly, As can

build up in the soil environment because of its strong binding affinity for quartz and aluminosilicates, which could facilitate subsequent contamination of aquatic and terrestrial ecosystems through remobilization. Hence, although what As binds to in various abiotic and biotic compartments controls a large portion of its behaviour in the environment, soil fertility amendment with poultry litter requires monitoring and regulation.

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