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Research Paper

Heavy Metal Analysis and MicrobiologicalCharacterization of Composted Human ExcretaDerived Fertilizer

[1]Sani Mohammed, [2]Buhari Idris, [1]Muhammad MalamiUsman, [2]Imrana Bello, [3]Umar Yahaya Dan Illela
[1]Department of Chemistry, ShehuShagari College of Education, Sokoto State, Nigeria
[2]Department of Chemistry, ShehuShagari University of Education, Sokoto State, Nigeria
[3]Department of Chemistry Kebbi State Polytechnic DakingariKebbi State, Nigeria

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Abstract
The continuous use of inorganic fertilisers poses a threat to soil fertility and ecosystem stability. The use of human excreta-derived fertiliser is at the forefront of sustainable agriculture, delivering benefits such as improved crop output and soil health. However, one of the most significant barriers to HDEF adoption is psychological stigma caused by sickness anxiety. The purpose of this study is to analyse the quality of three composts made from human excreta fertiliser by analysing their microbiological properties and metal pollution risk. The heavy metal concentrations of the composts were as follows: Zn > Cu >Pb> Cr > Ni. Their concentrations were determined to be within permitted limits. Bacteria such as Micrococcus flavus, Micrococcus varians, Pseudomonas aeruginosa, Rhizobium leguminosarum, Bacillus subtilis, Xanthomonascampestris, Clostridium perfringes, Alcaligenesfaecalis, and Staphylococcus aureus were identified in the compost. Our data show that the composts had lower metal toxicity, however additional methods to reduce the compost's hygiene risk should be addressed.

***Keywords**: Composted Human Excreta Derived Fertilizer, heavy metal, bacterial isolates, soil fertility*

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I. Introduction

Growing concerns about future fertiliser availability have re-emphasized the need for better nutrient management, including comprehensive recycling of nutrients contained in human excreta to agriculture (Dawson & Hilton, 2011; McConville et al., 2015). A circular approach to agriculture through the recovery and reuse of waste materials is important for sustainable agricultural development. The recovery of mineral elements from human excreta and their use in agriculture are now top priorities worldwide due to rapid population growth, urbanisation, and nutrient mining, as well as the need to feed the world's expanding population (Harder et al., 2019).

The long-standing tradition of using human waste as fertiliser and an organic soil enhancer has been significantly disrupted by the increasing and pervasive use of synthetic fertilisers (Ferguson, 2014). In Nigeria and many other African nations, applying fertiliser made from human faeces to land is a popular technique that permits the recycling of nutrients for agricultural use. Concerns regarding impurities including viruses, organic pollutants, and heavy metals in the generated fertiliser, however, have caused the procedure to be hotly disputed for a long time (Oberg & Mason-Renton, 2018).

Good agricultural practices, such as conservation farming and the use of organic matter, can aid in restoring soil health (Sasmal and Weikard, 2013). However, developing countries, particularly in sub-Saharan Africa, continue to struggle with degraded soils and low fertilizer usage (Druilhe and Barreiro-Hurlé, 2012). According to Sheahan and Barrett (2017), the average fertilizer application is only 26 kg per hectare, highlighting the necessity for alternative fertilizer sources. The Sustainable Development Goals (SDGs) include a focus on clean water and sanitation in Goal 6, while Goal 12 addresses responsible production and consumption to reduce waste (SDG, 2015).

Human waste has a high nutrient value (Ngone et al., 2018) and a growing movement of farmers, sanitation engineers, and scholars see the use of human waste as an alternative method for agricultural fertilization on a planet with dwindling resources (Cheng et al., 2017). The application of human excreta derived fertilisers (HEDF) may help address the problems of faecal sludge management and low soil fertility particularly in highly populated areas. However, there is a lot of stigma associated with fertilisers made from human waste and unclear laws for its application in agriculture. Thus this study aimed to assess heavy metal and Microbiological content of composted human excreta derived fertilizer from some selected villages in Sokoto State, Nigeria.

II. Material and Methods

Sampling and Samples Preparation

The sampling was done in accordance with the method reported by Kostadinova*et al*, (2022) with some modification. The compost samples were collected after 21 days maturation period. Approximately 100g of compost samples were taken from the 3 sites. The spot sample was taken at 5 cm from the surface and at 20 cm from the bottom. The samples were collected in a plastic bags and transported for analysis

Heavy Metal Analysis

The detection of heavy metals (cadmium, lead, zinc, copper, nickel, chromium), were carried out using atomic absorption spectrophotometer (Agilent technologies USA, model 240FF) the compost samples were dried at 105ºC and then digested in a mixture of (5:1 v/v) of di-acid (HNO_3 and perchloric acid) solution following APHA, 2005 standard procedures.

Bacterial Isolation

Serial dilutions of the bacterial suspension were prepared and loopful of the dilutions were streaked to sterilized semi-selective growth medium composed. The streaked Petri dishes were incubated in an inverted position at 37°C for 48 h to allow colonies to grow. Sub-culturing was carried out by taking loopful of the bacterium directly from separate and uniform colonies in growth plates. Identification was made based on colony characteristics. Colonies counting were done manually (Yemata*et al.,* 2019)

Statistical Analysis

Statistical analysis were performed using Excel 2010 (Microsoft OfficeStandard). The occurrence of a linear correlation between analyzed variables was evaluated by Pearson'scorrelation coefficient (r).

III. Result and Discussion

Table 1: Heavy meta analysis of the composted human faecal

METALS	DANMADI (D)	KAYA (K)	TAMBUWAL (T)	FCO
Nickel (Ni)	4.250	ND	6.700	50
Chromium (Cr)	6.800	12.225	10.250	50
Cadmium (Cd)	ND	ND	ND	5
Zinc (Zn)	478.150	646.275	326.900	1000
Lead (Pb)	30.925	24.125	31.225	100
Copper (Cu)	101.125	117.375	68.000	300

FCO; Fertilizer Control Order of India (FCO, 2003) (Source; Mehvish*et al.,* 2021)

The results of heavy metal analysis is presented in Tables 1 and Fig.1, It can be observed from the table that the concentrations of heavy metal of the compost from Kaya village exceeded that of Dan-madi and Tambuwal but were all within the prescribed fertility control order standards (FCO). According to Ding et al. (2017), there are varying amounts of heavy metals from food additives in farm manure and sludge. Additionally, Ding et al. (2017) discovered that these metals' concentrations in composts differ significantly depending on the source and the location. The mean levels of heavy metals in the compost; Ni, Cr, Cd, Zn, Pb and Cu were 3.65, 9.76, 0, 483.78, 28.76, and 95.5 mg/kg, respectively. Zn, Pb and Cu were found to be the most dominant heavy metals in all the samples, while Cd and Ni were the least. The above observation is in good agreement with the results obtained by other researchers (Turek et al., 2019, Álvarez et al., 2002, Ji et al., 2015). Furthermore, it should be noted that excessive levels of Cd and Hg are extremely harmful to both people and animals, whereas Zn and Cu have the opposite effect (Tiruneh et al., 2014). According to Abdullah (2022), dietary additives that prevent sickness contains: As, Co, Cu, Fe, Mn, Se, and Zn; this could be the cause of the composts' highest zinc content. According to reports, zinc is the most prevalent heavy metal in sewage sludge and swine manure, and it was the most prevalent metal in all of the composted samples examined (Hseu, 2004).

Excessive heavy metal content limits the application of compost to agricultural land. Heavy metal-contaminated compost has varying environmental effects depending on soil type, plant species, and compost quality (Zhao et al., 2011). Proper use of composts and fertilisers with low heavy metal concentration may reduce heavy metal accumulation in soils, reducing possible hazards to soil quality (Ding et al., 2017).

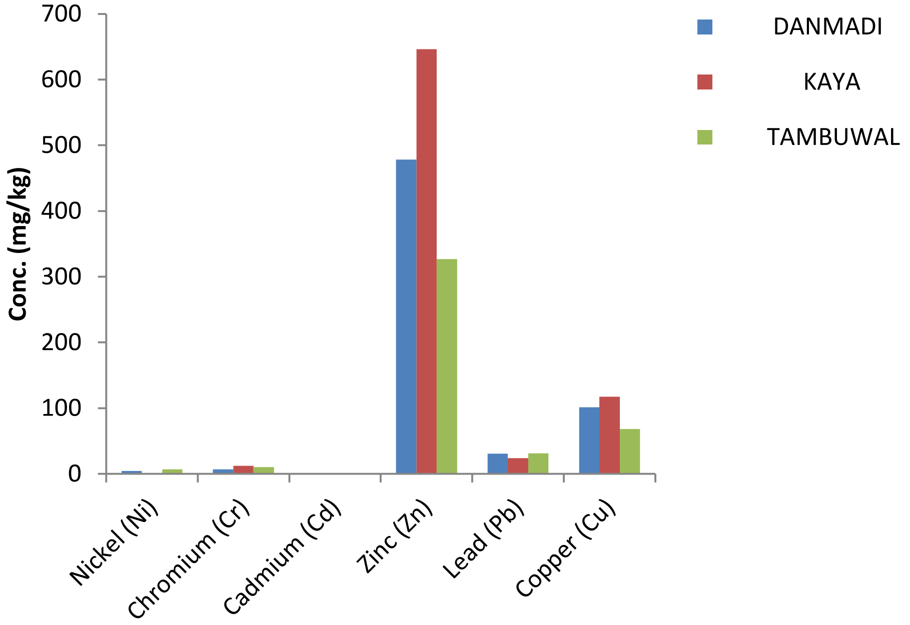

Figure 1: Graphical Heavy Metals

Table 2: Pearson Correlation Co-effecieant of the heavy metals

	Ni	Cr	Cd	Zn	Pb	Cu
Ni	1					
Cr	-0.4984	1				
Cd	0.0000	0.0000	1			
Zn	-0.9924	0.3879	0.0000	1		
Pb	0.9453	-0.7539	0.0000	-0.8979	1	
Cu	-0.9398	0.1722	0.0000	0.9747	-0.7769	1

The results of the Pearson's correlation for the heavy metals detected in the samples is shown in (Table 2).The results indicate neagativecorrelationsbetween the elementsat 0.05% confidence level,with exception of copper vs lead andnickelvslead which shows strong positive correlation. Negative correlations between the heavy metals may prove that,they possibly have different accumulation behavior or originate from variant sources.

Characterization of the bacterial isolates.

The colony characteristics of the isolates werepresented in Table 3. The isolates were small and large colonies with smooth and rough edges. The color of the colonies was mostlywhite. Six of the isolates were Grampositive and these were rods and cocci. Gramnegative character were exhibited by three isolates with rod cell shapes. The colonycount of the bacterial isolates is shown in Fig. 2.Dan-madi compost shown high count of the isolates compare to those from Kaya and Tambuwal.

Table 3: Colony Characteristicsof the Isolates

Colony No	Shape	Color	Gram character	Shape of the cell
K_1	Large colony with smooth edge	White	Gram positive	Cocci
K_2	Large colony with rough edge	White	Gram positive	Cocci
K_3	large colony with rough edges	Yellow	Gram positive	Rod
T_1	Large colony with smooth edge.	White	Gram negative	Rod
T_2	Small colony with rough edge	White	Gram positive	Rod
T_3	Large colony with smooth edge	Yellow	Gram negative	Rod
D_1	Large colony with smooth edge	White	Gram positive	Rod
D_2	Large colony with rough edge	Yellow	Gram negative	Rod
D_3	Large colony with rough edge	White	Gram positive	Cocci

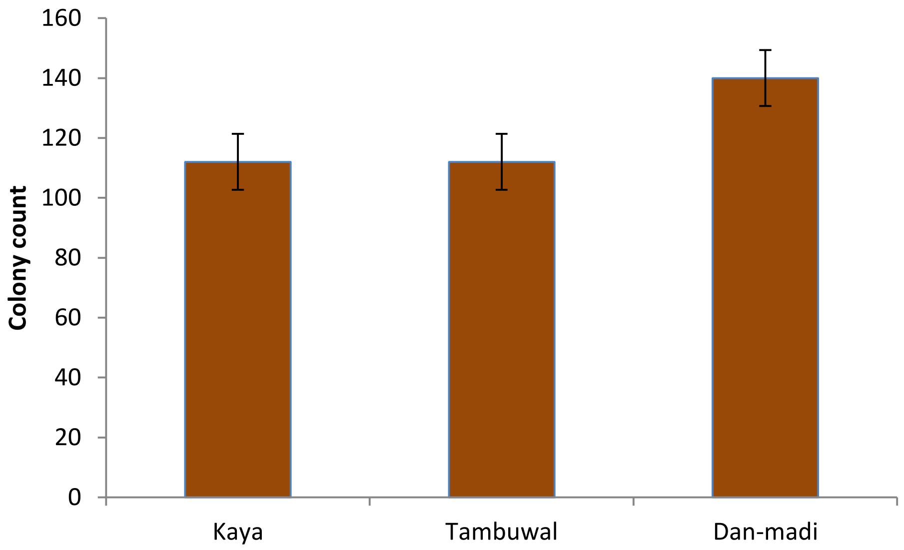

Figure 2: ColonyCount of the Bacterial Isolatesfrom the Composts

Table 4: Various Biochemical Characteristics of theIsolated Bacteria.

Samples	Mic.	Cit.	Mr	ind	glu	suc	Lac.	H_2s	mo	Hae	Star	Urea	VP	Oxi	Cat.	Coa
Kaya	Gram +vecocci	–	–	–	-	–	–	–	–	–	–	–	–	–	+	+
Kaya	Gram +vecocci	–	–	–	+	–	–	-	–	–	–	–	–	–	+	+
Kaya	Gram +ve rod	–	–	–	–	-–	+	–	+	–	–	–	–	+	–	–
Tambuwal	Gram -ve rod	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-
Tambuwal	Gram +ve rod	+	-	-	+	+	+	-	+	-	+	-	+	-	+	-
Tambuwal	Gram -ve rod	-	-	+	+	+	-	+	-	-	+	-	-	-	+	-
Dan-madi	Gram +ve rod	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-
Dan-madi	Gram -ve rod	+	-	-	-	-	-	-	+	+	-	-	-	+	+	-
Dan-madi	Gram +vecocci	+	+	-	+	+	+	-	-	-	-	+	+	-	+	+

Key: SA – sample, Mic – microscopy, Cit – Citrate, Mr – Metyl red, Ind – Indole, Glu – Glucose, Suc – Sucrose, Lac – Lactose, Mo – Motility, Hae – Haemolysis, Star – Starch, Urea – Urease, Ox – Oxidase, Cat – Catalase, Coa – Coagulase

Table 5: Organism Isolated from the Samples

Colony No	Organism
K_1	*Micrococcus flavus*
K_2	*Micrococcus varians*
K_3	*Pseudomonas aeruginosa*
T_1	*Rhizobium leguminosarum*
T_2	*Bacillus subtilis*
T_3	*Xanthomonascampestris*
D_1	*Clostridium perfringes*
D_2	*Alcaligenesfaecalis*
D_3	*Staphylococcus aureus*

The compost samples were tested for the presence of some pathogens. Pathogens like *Salmonella* and Coliform and *E. coli* were not found in the compost. Different organisms were detected in the tested samples, this could be due to variations in the sites and/or soil. The compost from Dan-madi shows the presenceof *Clostridium perfringens, Alcaligenesfaecalis and Staphylococcus aureus. Clostridium perfringens (C. perfringens)* is a Gram-positive, obligate anaerobic, spore-forming, non-motile bacillus that is ubiquitous in the normal microbiota of the gastrointestinal tract of humans and animals, as well as in the environment, including food, wastewater, and soil (Li et al., 2013). C. perfringens is highly resistant to adverse environments as it can form spores and biofilms, which are highly prevalent in humans, chickens, and mammals (Yadav et al., 2017; Milton et al., 2017). It is the main agent of foodborne diarrhea and gas gangrene in humans.

Staphylococcus aureus is a gram-positive, cocci-shaped species in the *Staphylococcaceae* family. This bacterium is frequently encountered in the environment and is part of the normal human microbiota, particularly on healthy skin and mucous membranes (DeLeo et al., 2010). It can lead to minor, serious, or even fatal infections under certain conditions, including minor skin and soft tissue infections, foodborne illness, and severe diseases like bacteremia and infective endocarditis (Tong et al., 2015). Alcaligenesfaecalis (A. faecalis) is a Gram-negative, obligate aerobic, nonfermenting bacterium typically found in soil and water. It poses a potential risk as an emerging pathogen that can cause opportunistic infections in humans. A. faecalis has been identified in various clinical samples, including urine, blood, wound fluids, stool, cerebrospinal fluid, and respiratory secretions (Mordi et al., 2013; Mordi et al., 2015; Siddiqui et al., 2015). A. faecalis is linked to various infections including endocarditis, bacteremia, meningitis, endophthalmitis, skin and soft tissue infections, urinary tract infections, otitis media, peritonitis, and pneumonia (Hasan et al., 2019; Al-Zakhari et al., 2020; Majewski et al., 2020). Treating A. faecalis infections can be challenging because of its heightened resistance to multiple antibiotics (Junejo et al., 2018; Chua et al., 2019).

Pseudomonas aeruginosa, Micrococcus flavus and varians were isolated in the compost from Kaya village. *Pseudomonas aeruginosa*This is a gram-negative aerobic bacterium found in various environments, such as soil, plants, and mammalian tissue (Stover et al., 2000). It can thrive in water and on various surfaces due to its effective binding mechanisms, including flagella, pili, and biofilms. Pseudomonas aeruginosa is commonly found in both natural and man-made settings, including lakes, hospitals, and household sink drains (Stover et al., 2011). As an opportunistic pathogen, it is responsible for various infections in humans and has emerged as a significant contributor to hospital-acquired infections and antibiotic resistance (Shariati et al., 2018; DeLeo et al., 2010).

Micrococci belong to the Micrococcaceae family and are typically viewed as contaminants from skin and mucous membranes. They can also be found in various environmental settings, such as water, dust, and soil. While Micrococcus is mainly considered a saprotrophic or commensal organism, it can act as an opportunistic pathogen, particularly in individuals with weakened immune systems, like those with HIV. Despite this, they have been identified as the cause of several infections, including bacteremia, endocarditis, ventriculitis, peritonitis, pneumonia, endophthalmitis, keratolysis, and septic arthritis (Selladurai et al., 1993; Smith et al., 1999; BBC News, 2016).

Bacillus subtilis is a rod-shaped, Gram-positive bacterium that primarily resides in soil. While it was previously thought to be an obligate aerobe, it has recently been reclassified as a facultative anaerobe.B. subtilis is motile due to its flagella, in a liquid environments and under harsh conditions. Additionally, it has the ability to form endospores, which contributes to its widespread presence in nature (Piggot 2009). B. subtilis is commonly found in the human gastrointestinal tract in a non-pathogenic state (Piggot et al., 2009). However, studies indicate that in neonates with weakened immune systems, it can lead to serious infections (Lampropoulos et al., 2021). Infections associated with B. subtilis, such as septicemia, pneumonia, endocarditis, wound infections, and intraocular inflammation, have been documented, along with cases of meningitis

(Ozkocaman et al., 2006). Infections caused by B. subtilis in humans are rare and typically occur in individuals with pre-existing health issues or compromised immune systems.

Xanthomonascampestris is a gram-negative bacterium that requires oxygen to survive and belongs to the Xanthomonas genus, a group primarily recognized for its links to plant diseases. These bacteria can act as facultative saprophytes, enabling them to thrive on dead or decaying organic material when conditions are suitable (Tanng et al., 2021). Traditionally, the Xanthomonas genus was thought to exist solely in association with plants and was not known to persist in other habitats (Darrasse et al., 2013). However, during a study examining the bacterial diversity found in the human microbiota through culturomics (Lagier et al., 2016), a strain of Xanthomonas was discovered in a stool sample from an obese patient in France.

Conclusion

Our research found that the composts contained a diverse range of microbial isolates, primarily opportunistic pathogens, and some were beneficial for soil enrichment and had agricultural significance. Each compost examined contained some amounts of chromium, lead, copper, nickel, and cadmium within acceptable limits, although lead levels were notably higher across all the waste composts. Consequently, regular heavy metal analysis and microbial risk assessments should be conducted when applying human excreta-derived fertilizer in agricultural settings to identify potential pollution risks. This approach could lead to the establishment of new regulations to mitigate the hazards associated with using such fertilizers to enhance soil fertility.

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