PHYTOTOXICITY ASSESSMENT OF GREEN AMARANTH (AMARANTHUS VIRIDIS LINN.) GROWN ON SOIL AMENDED WITH SEWAGE SLUDGE

Taofik A. Towolawi[1]*, Toyin A. Arowolo[1], T. Babatunde S. Bada[1], Adekunle A. Badejo[2], Adewale M. Taiwo[1]

[1]Department of Environmental Management and Toxicology
[2]Department of Civil Engineering

Federal University of Agriculture, Abeokuta, P.M.B 2240, Ogun State, Nigeria

taofiktowolawi@yahoo.com
Tel: +2348060172946

ABSTRACT

Sewage Sludge (SS) land application remains the best sustainable disposal method. The present study evaluated SS samples tested on *Amaranthus viridis* Linn for phytotoxic effects. The soil and SS samples were analysed for Organic Matter (OM), N, P and K contents; exchangeable cations: Na, Ca and Mg using standard methods. Application of the SS followed N-requirement of the test plant in a completely randomised design with three replicates. Plant growth parameters measured for phytotoxicity test were germination, stem diameters, number of leaves and height. The shoot of the plant was analysed for Cr, Zn, Cu, Cd and Pb using Atomic Absorption Spectrophotometer (AAS). Data collected were analysed for simple descriptive and inferential statistics. Range of results for analysed parameters in SS samples before treatments were OM = 384.20 ± 12.40 to 576.50 ± 3.00 g kg^{-1}, N = 9.10 ± 0.53 to 19.60 ± 0.53 g kg^{-1}, P = 11.98 ± 0.95 to 22.74 ± 0.65 mg kg^{-1}, K = 5.13 ± 1.39 to 10.26 ± 0.25 cmol kg^{-1}. The plant had steady growth parameters. Most of the analysed metals were below detection limits in the shoot except for Zn that ranged from 1.43 ± 0.23 to 3.88 ± 0.30 mg kg^{-1}. The SS amended soil exhibited no phytotoxic effects on plant growth.

Keywords: Sustainable disposal, N-requirement, Germination, FUNAAB, Nigeria

INTRODUCTION

Having ascertained phytoaccumulation of *Amaranthus viridis* Linn in our previous study [1], there was then the necessity for phytotoxicity. Phytotoxicity is the poisonous effect to plant with haphazard germination as a result of pollutants in an amendment, causing short-term or permanent damage to plants [2]. Phytotoxic effects could be an indicator of toxicity to the plant when applied as amendments to improve soil fertility. The adverse effect occurs on plant growth parameters [3]. Adverse health risks occur as a result of bioaccumulation of toxicants such as heavy metals (HMs) in the food chain [4] when amendment augments agricultural land. The most occurring symptoms exhibited by plants grown on polluted amended soil include chlorosis, necrosis, roots and

*Corresponding author

leaves reduction. Such symptoms of toxicity vary in a plant when high level of HMs bio-accumulate above the threshold [5]. Efficacy of HMs bioaccumulation varies with sources and composition of amendments, and the processes adopted in the amendment preparation [6]. Acidic pH (< 5.2) influences high HMs contents. A typical amendment is the sewage treatment byproduct (sludge), which after biodegradation releases carbon dioxide, ammonia and water, and becomes a sturdy organic material that could improve agricultural soil fertility [7]. Several studies had sustainably utilised the sludge when it is undesirable to discharge the byproduct in an unfriendly manner [1; 8]. Enrichment nature of SS with high organic matter and essential nutrient contents could influence algal blooms if the material gets eroded into water bodies. This enhances proliferation of microbial loads in the environment. Consequently, sources of potable water for human consumption would be polluted and increase competition on the available water. Furthermore, bioaerosols are released into the air, thereby posing health risk effects on human. The health risks can occur as a human comes in contact with the bioaerosol through inhalation and ingestion with various respiratory, gastrointestinal and dermal infections. The best abatement measure is to curb widespread of SS in the surroundings by adopting sustainable utilisation.

Sewage Sludge (SS) application benefits include but not limited to alternative disposal method [9], increase land fertility reclamation [7], provide soil nutrients [10], and enhance plant biomass [11]. SS could improve agricultural produce three times more than the capacity of commercial fertilisers [12]. It adds supplement capacity like NPK (nitrogen, phosphorous and potassium) fertiliser and liquid manure to the soil [13] and higher contents of ammonium than liquid manure owing to carbon losses during anaerobic digestion, which may result in narrowing the ratio of calcium to nitrogen [14]. The possibility is when such SS undergoes the process that reduces pathogens, odour and inhibits putrefaction, which is useful for crop production and soil health improvement [15]. Kumar and Chopra [16] observed that increasing application of SS led to an increase in various metal accumulations in roots, shoots, leaves and fruits. The SS could contain toxic metals, but the level and time that such metals induce adverse effects are rarely and hardly known. Wastewater treatment does not absolutely ascertain total removal of pollutants, especially the HMs contents. Secondary pollution in the environment is thus inevitable. Speciation forms and interactions along the pathway within the natural environment of the HMs dictate levels and extent of environmental toxicity elicited by the HMs. The SS is environmentally beneficial when its HMs contents do not exceed the threshold [17]. Safe utilisation of SS would, therefore, depends on the HMs contents available in the soil to be amended, and the soil pH monitored for conformity to the local and international standards. A previous report [18] observed that application of SS for soil amendment carried low risks if HMs contents are within limits in both SS and soil.

Sewage sludge rarely poses phytotoxic effects on plants as reported in studies from North America [19], South Africa [20], Nigeria [21], Brazil [22], and Mexico [23]. SS-based fertilisers had been applied [24], because of their high biogenic (such nitrogen and phosphorus) composition required for optimal growth of plants. One of the plants that thrive on garbage is Amaranth. Amaranths grow on well-drained alkaline (pH > 6) and loose structure soils rich in nitrogen. However, a high level of nitrogen influences and delays the onset of flowering and low leaf yields [25]. The plant has high mineral uptake potential and reaches harvest between 4 and 5 weeks after sowing. The whole amaranth is used as medicine to heal different diseases in African communities [26]. Improvement of amaranths through advanced research and development could produce easy and cost-effective ways of eliminating malnutrition and promoting people's health as well as achieving food security [27]. The advanced research would develop physiological responsive measures in the amaranths, while development would work on the available methodologies for the sustainability various species of amaranths to cope with likely changes imposed by the non-essential contents of the amendments. A study on four *Amaranthus* species (*A. blitum*, *A. spinosus*, *A. tricolor* and *A. viridis*) showed that the highest amount of proteins, carbohydrates and calcium were found in *A. spinosus*, while *A. viridis* contained maximum amount of iron and sodium [28]. The study applied SS as soil amendment using N-requirement of *A. viridis* (67.5 kg N ha^{-1}) [29] and increased quantities to test for phytotoxic effects and optimal N-requirement. This study aimed to find a sustainable and environment-friendly solution to the disposal of sewage sludge.

MATERIALS AND METHODS

Sewage sludge samples collection

Sewage sludge (SS) samples were collected from three waste treatment facilities in Abesan (SSA), Oke-Afa (SSO) and Ikeja (SSI) under the supervision of Lagos State Wastewater Management Office, Obasa, Ikeja, Nigeria. The facility is in Alimosho, Oshodi-Isolo and Ikeja Local Government Areas, respectively. Map of the study areas is presented in Figure 1.

Seed and soil samples collection

Seeds of *Amaranthus viridis* were collected from the National Horticultural Institute, Ibadan, Oyo State, Nigeria. Soil samples (topsoil of 0-15 cm depth) were taken from the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria.

Samples preparation and SS application

Soil and SS samples were air-dried, sieved through a 2 mm mesh for homogeneity. Soil sample (5 kg) measured in thirty-six plastic pots of 7 L capacity. They were all perforated underneath, placed on their covers each followed by application of SS, watered and allowed to equilibrate for 21 days before planting for mineralisation. The drained water was returned into each pot to avoid nutrient loss [30]. The *A. vi-*

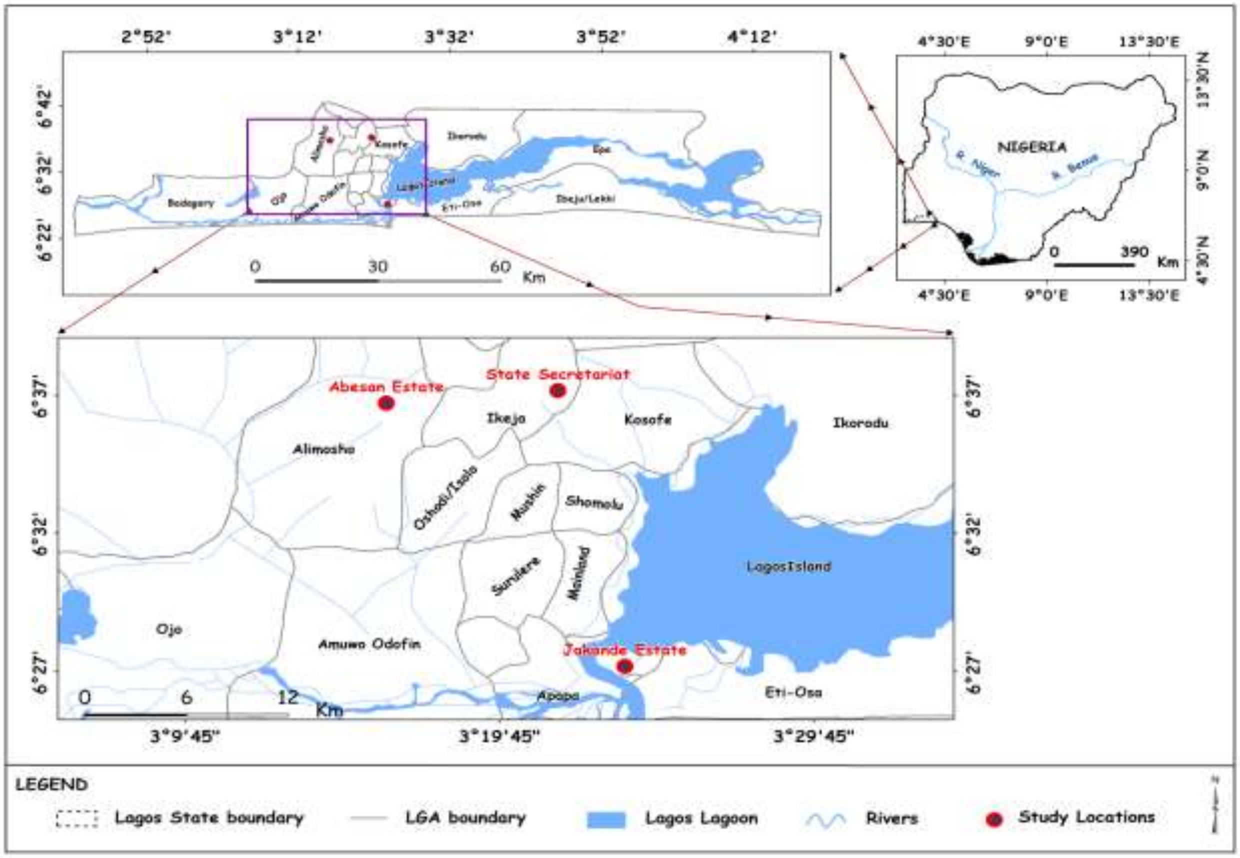

FIGURE 1
Map of the sewage sludge sampling areas in Lagos State

ridis Linn seeds were planted in each bowl and thinned when started growing to ensure healthy germination and prevent competition for nutrients. Imported organic fertiliser (Pupuk) and NPK (20:10:10) were the other amendments used aside SS.

The experiment took place in the FUNAAB screen house. The pots arranged in Completely Randomized Design (CRD) with three replicates. The treatments were SSA, SSI, SSO, Imported organic fertiliser (Pupuk), NPK and the Control. The SS samples applied at 67.5, 87.5 and 107.5 kg N ha^{-1} while Pupuk and NPK (20:10:10) at 67.5 kg N ha^{-1} as the N-requirement of the test plant [31].

Laboratory analyses

Standard methods analysed soil and SS samples for various parameters. Particle size distribution was determined by Buoyancies' method [32], pH in water suspension using a 1:2.5 soil: solution ratio (w:w) after 30-min equilibrium time [33]. Wet dichromate acid oxidation method determined Organic Carbon (OC) content [34] and multiplying OC value by 1.72 gave the organic matter content [29; 30]. Micro-Kjeldahl digestion-distillation procedure was adopted to determine total nitrogen [35], while available phosphorus was analysed by Olsen method [36]. Ammonium acetate method was used to extract the Ca, Mg, Na and K (1 N NH_4OAC at pH 7.0) [23]. Both Na and K were determined with Flame Emission Photometer (Model PFP7, Jenway Ltd, UK), while Ca and Mg were measured titrimetrically [30]. The shoot (leaves and stems) of the test plant were analysed for five selected HMs (Zn, Cu, Cd, Cr and Pb) [11; 37] using Atomic Absorption Spectrometer (Buck Scientific, Model 210VGP, CT, USA) after being washed, air dried and ground with pre-cleaned mortar and pestle. The ratio of nitric, sulphuric and perchloric acids mixture for digestion was in 5:1:2 [38].

Phytotoxicity test

Continuous monitoring of *A. viridis* was done weekly for six weeks [25] when it was between 15 and 20 cm height [29] after planting to observe its growth parameters using a measuring rule for plant height, calliper for stem diameter and manual counting for the branch leaves. Manual count was adopted for germinated plants per pot, and weighing balance gave plant biomass at harvest after washing with distilled water [30].

Data analysis

The obtained data were subjected to descriptive (mean and standard deviation) and inferential (ANOVA and Duncan's Multiple Range Test) statistics using SPSS for Windows version 22.0.

RESULTS AND DISCUSSION

Results of the soil and sewage sludge samples analyses before treatments

Results of the soil were organic matter (OM) = 61.77 ± 1.36 mg kg^{-1}, N = 1.40 ± 0.12 g kg^{-1}, P = 7.35 ± 0.05 mg kg^{-1}, K = 2.56 ±0.05 Cmol $kg^{-1}$and C/N = 26:1 (Table 1). The soil was low in nutrients compared to the soil fertility rating classes in Nigeria [37] and might be due to continuous cultivation of the land and inorganic fertiliser application [30]. Low level of soil OM conformed to the value (59.67 – 61.61 %) observed by Vaca *et al.* [23], who said the low level could have resulted from microbial decomposition leading to CO_2 emission [39], reduction in ammonia and moisture contents. The data of SS parameters are presented in Table 2. The nutrient enrichment of the SS proved its usefulness and made the SS samples suitable to augment the low soil nutrients [40]. The OM, N, P, K and Mg except for pH and Ca determined in this study were higher than those that were earlier reported by

TABLE 1
Results of soil analysis

Parameter	Soil
pH	7.78 ± 1.99
Sand (g kg^{-1})	970.00 ± 26.48
Clay (g kg^{-1})	0.00
Loam (g kg^{-1})	30.00 ± 1.00
OM (g kg^{-1})	61.77 ± 1.36
N (g kg^{-1})	1.40 ± 0.12
C:N	26:1
P(mg kg^{-1})	7.35 ± 0.05
Na (Cmol kg^{-1})	4.35 ± 0.05
K (Cmol kg^{-1})	2.56 ± 0.05
Ca (Cmol kg^{-1})	0.50 ± 0.00
Mg (Cmol kg^{-1})	0.82 ± 0.02

TABLE 2
Results of the SS samples analysis

Parameters	SSA	SSI	SSO
pH	6.46 ± 0.20^a	6.40 ± 0.10^a	7.16 ± 0.53^b
Sand (g kg^{-1})	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a
OM (g kg^{-1})	384.20 ± 12.40^a	576.50 ± 3.00^c	453.00 ± 2.60^b
N (g kg^{-1})	19.60 ± 0.53^c	9.10 ± 0.53^a	17.50 ± 1.50^b
C:N	1:1	4:1	2:1
P(mg kg^{-1})	16.90 ± 0.85^b	11.98 ± 0.95^a	22.74 ± 0.65^c
Na (Cmol kg^{-1})	8.70 ± 0.40^a	17.40 ± 1.15^c	13.04 ± 0.07^b
K (Cmol kg^{-1})	5.13 ± 1.39^a	10.26 ± 0.25^c	7.70 ± 0.82^b
Ca (Cmol kg^{-1})	1.40 ± 0.10^a	1.90 ± 0.10^b	5.25 ± 0.31^c
Mg (Cmol kg^{-1})	2.14 ± 0.14^b	1.52 ± 0.02^a	4.12 ± 0.07^c

Superscripts with the same letters across the row were not significantly ($p < 0.05$) different (DMRT).
SS= sewage sludge; A= Abesan; I= Ikeja; and O= Oke-afa

Antonious and Snyder [41]. The SS amended soil improved OM, P and N contents when compared with NPK-amended soil [23].

Effects of sewage sludge application on Green amaranth growth (Phytotoxicity Test)

Effects of sewage sludge amendments on germination. Soil or soil additive is not posing adverse effects on the plant sown on it when the plant grows. The more the plant grows, the more favourable the soil or additive is. *A. viridis* germinated the 1st WAP in all the pots, and the number of sprouted plants varied from ~4.00 ± 1.00 in 107.5 kg SSI, 67.5 kg SSO, 87.5 kg SSO and 67.5 kg NPK to ~6.00 ± 0.58 in 107.5 kg SSA on average. In the 2nd WAP, the number of plants germinated increased ranging from ~4.00 ± 1.16 in 87.5 kg SSO to ~8.00 ± 0.58 in 107.5 kg SSA. In the 3rd WAP, 87.5 kg SSA setup influenced ~10.00 ± 0.58, while the Control impacted ~7.00 ± 1.16 numbers of germinated plants. The number of grown plants with a range from ~8.00 ± 0.58 in the Control to ~10.00 ± 0.00 in the 87.5 kg SSA with no significant differences ($p > 0.05$) in all the treatments occurred from the 4th to 6th WAP (Table 3).

Effects of sewage sludge amendments on stem diameter. The plant sprouted a WAP with highest diameters of 1.32 ± 0.55 mm in 107.5 kg SSI, 87.5 kg SSO and 67.5 kg NPK with the lowest diameter of 0.80 ± 0.00 in 67.5 kg SSI. In the 2nd WAP, 2.03 ± 0.06 mm and 2.30 ± 0.52 mm were the least and highest diameter in 67.5 kg Pupuk and 87.5 kg SSA. In the 3rd WAP, diameter increment was the highest with a value of 3.06 ± 0.10 mm in 87.5 kg SSI and least with a value of 2.22 ± 0.38 mm in 87.5 kg SSO significantly. Plant diameter increments ranged from 3.03 ± 0.06 mm in 67.5 kg Pupuk to 3.30 ± 0.52 mm 87.5 kg SSA in the 4th WAP with no significant difference ($p > 0.05$). Increments in plant diameter were measured to range from 3.08 ± 0.14 mm and 3.11 ± 0.19 mm in 67.5 kg Pupuk to 5.30 ± 0.06 mm and 5.19 ± 0.33 mm in 87.5 kg SSA in the 5th and 6th WAP significantly ($p < 0.05$) (Table 4).

Effects of sewage sludge amendments on leaves. The number of *A. viridis* leaves was counted to range from ~2.00 ± 0.23 in 107.5 kg SSO to ~5.00 ± 0.27 in 107.5 kg SSI in the 1st WAP. It ranged from ~4.00 ± 0.10 in 67.5 kg SSO to ~7.00 ± 0.20 in 107.5 kg SSI in the 2nd WAP. The number of leaves counted on the 3rd WAP ranged from ~5.00 ± 0.09 in 107.5 kg SSO to ~8.00 ± 0.29 in 107.5 kg SSI while in the 4th WAP, it was between ~6.00 ± 0.12 in 87.5 kg SSO and ~8.00 ± 0.28 in 67.5 kg SSA. The count ranged from ~8.00 ± 0.15 in 107.5 kg SSI to ~11.00 ± 0.29 in 87.5 kg SSI in the 5th WAP, while further counting was observed to range from ~10.00 ± 0.33 in 107.5 kg SSO to ~13.00 ± 0.29 in 87.5 kg SSA in the 6thWAP. There were significant ($p < 0.05$) differences in the number of counted leaves throughout the growing period of the plant (Table 5).

Effects of sewage sludge amendments on height. In the 1st WAP, the height of *A. viridis* was highest: 2.33 ± 0.76 cm in 67.5 kg NPK and least: 1.43 ± 0.60 cm in 67.5 kg SSI. In the 2nd WAP, least and highest values were 2.83 ± 0.91 in 67.5

TABLE 3
Effects of sewage sludge amendments on germination

Soil Amendments	Week After Planting (WAP)					
(kg N ha^{-1})	1st	2nd	3rd	4th	5th	6th
67.5 SSA	4.33±0.58^{ab}	4.67±0. 0.58^{ab}	8.33±0.58^{ab}	9.00±1.00^{a}	9.00±1.00^{a}	9.00±1.00^{a}
87.5 SSA	5.33±0.58^{ab}	6.67±0.58^{cde}	9.67±0.58^{b}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}
107.5 SSA	5.67±0.58^{b}	8.33±0.58^{e}	9.33±0.58^{b}	9.33±0.58^{a}	9.33±0.58^{a}	9.33±0.58^{a}
67.5 SSI	4.67±0.58^{ab}	6.33±0.58^{bcd}	8.33±0.58^{ab}	9.00±1.00^{a}	9.00±1.00^{a}	9.00±1.00^{a}
87.5 SSI	5.00±1.00^{ab}	6.67±1.44^{cde}	9.00±1.00^{ab}	9.00±1.00^{a}	9.00±1.00^{a}	9.00±1.00^{a}
107.5 SSI	4.00±1.00^{a}	7.33±0.58^{de}	8.33±0.58^{ab}	9.00±1.00^{a}	9.00±1.00^{a}	9.00±1.00^{a}
67.5 SSO	4.00±1.00^{a}	5.00±2.00^{abc}	8.67±0.58^{ab}	9.33±0.58^{a}	9.33±0.58^{a}	9.33±0.58^{a}
87.5 SSO	4.00±1.00^{a}	4.33±1.16^{a}	8.33±0.58^{ab}	8.67±0.58^{a}	8.67±0.58^{a}	8.67±0.58^{a}
107.5 SSO	5.00±1.00^{ab}	7.33±0.58^{de}	8.67±2.08^{ab}	9.33±1.53^{a}	9.33±1.53^{a}	9.33±1.53^{a}
67.5 NPK	4.00±1.00^{a}	7.33±0.58^{de}	8.00±0.00^{ab}	8.67±1.16^{a}	8.67±1.16^{a}	8.67±1.16^{a}
Control	4.33±0.58^{ab}	6.67±1.16^{cde}	7.33±1.16^{a}	8.33±0.58^{a}	8.33±0.58^{a}	8.33±0.58^{a}
67.5 Pupuk	5.33±0.58^{ab}	7.00±1.73^{de}	7.33±1.53^{a}	8.67±1.53^{a}	8.67±1.53^{a}	8.67±1.53^{a}

Superscripts with the same letters down the column were not significantly ($p < 0.05$) different (DMRT). SS= Sewage Sludge; A= Abesan; I= Ikeja; and O= Oke-afa

TABLE 4
Effects of sewage sludge amendments on stem diameter (mm)

Soil Amendments	Week After Planting					
(kg N ha-1)	1st	2nd	3rd	4th	5th	6th
67.5 SSA	1.12±0.20a	2.22±0.38a	2.28±0.49a	3.04±0.07a	3.32±0.55ab	4.09±0.16b
87.5 SSA	1.24±0.42a	2.30±0.52a	3.04±0.07b	3.30±0.52a	5.03±0.06cd	5.19±0.33c
107.5 SSA	1.24±0.42a	2.24±0.42a	3.00±0.00b	3.13±0.23a	3.32±0.56ab	4.10±0.18b
67.5 SSI	0.80±0.00a	2.17±0.29a	2.28±0.49a	3.19±0.32a	4.00±0.00bc	4.24±0.42b
87.5 SSI	1.17±0.29a	2.29±0.50a	3.06±0.10b	3.30±0.53a	4.31±0.54cd	5.18±0.32c
107.5 SSI	1.32±0.55a	2.22±0.38a	2.23±0.40a	3.08±0.14a	3.30±0.53ab	4.00±0.00b
67.5 SSO	0.82±0.03a	2.20±0.35a	2.28±0.49a	3.07±0.13a	3.32±0.56ab	4.03±0.06b
87.5 SSO	1.32±0.55a	2.05±0.09a	2.22±0.38a	3.07±0.12a	3.31±0.53ab	4.00±0.00b
107.5 SSO	1.17±0.29a	2.12±0.20a	2.25±0.44a	3.07±0.13a	3.32±0.56ab	4.06±0.10b
67.5 NPK	1.32±0.55a	2.27±0.46a	3.03±0.06b	3.23±0.40a	4.00±0.00bc	4.02±0.03b
Control	1.07±0.12a	2.17±0.29a	2.25±0.44a	3.05±0.09a	3.28±0.49ab	4.00±0.00b
67.5 Pupuk	1.00±0.00a	2.03±0.06a	2.25±0.44a	3.03±0.06a	3.08±0.14a	3.11±0.19a

Superscripts with the same letters down the column were not significantly ($p < 0.05$) different (DMRT).
SS= sewage sludge; A= Abesan; I= Ikeja; and O= Oke-afa

TABLE 5
Effects of sewage sludge amendments on number of leaves

Soil Amendments	Week After Planting					
(kg N ha^{-1})	1st	2nd	3rd	4th	5th	6th
67.5 SSA	3.13±0.14b	5.10±0.17b	7.13±0.13d	8.23±0.28c	9.17±0.29b	10.33±0.29a
87.5 SSA	5.13±0.13d	6.13±0.13c	7.30±0.36d	8.17±0.21c	10.17±0.18c	13.33±0.29d
107.5 SSA	3.33±0.29b	4.30±0.29a	6.20±0.20c	8.17±0.29c	10.17±0.15c	13.17±0.29d
67.5 SSI	4.17±0.18c	5.23±0.20b	5.30±0.27b	7.52±0.27b	9.17±0.29b	12.33±0.35c
87.5 SSI	4.47±0.43c	5.30±0.27b	6.30±0.27c	7.17±0.15b	11.33±0.29d	12.33±0.35c
107.5 SSI	5.20±0.27d	7.20±0.20c	8.17±0.29e	8.20±0.20c	8.17±0.15a	11.33±0.29b
67.5 SSO	4.32±0.28c	4.03±0.10a	4.30±0.26a	6.27±0.24a	9.30±0.27b	12.30±0.41c
87.5 SSO	2.33±0.29a	4.30±0.27a	5.20±0.18b	6.13±0.12a	8.30±0.32a	11.27±0.36b
107.5 SSO	2.20±0.23a	5.30±0.27b	5.10±0.09b	7.17±0.18b	8.30±0.33a	10.30±0.33a
67.5 NPK	4.30±0.27c	5.37±0.44b	6.30±0.30c	6.40±0.35a	10.30±0.27c	12.25±0.35c
Control	3.30±0.27b	4.40±0.36a	6.37±0.40c	7.30±0.36b	8.30±0.27a	10.30±0.30a
67.5 Pupuk	3.20±0.20b	5.10±0.10b	5.30±0.30b	7.20±0.17b	8.50±0.50a	10.97±0.45b

Superscripts with the same letters down the column are not significantly ($p < 0.05$) different (DMRT).
SS= sewage sludge; A= Abesan; I= Ikeja; and O= Oke-afa

kg Pupuk and 5.33 ± 1.91 cm in 87.5 kg SSA. In the 3rd WAP, the height was measured and varied from 3.67 ± 1.94 cm in 87.5 kg SSO to 7.00 ± 2.40 cm in 87.5 kg SSI. There was a further increment in the 4th WAP, and this ranged from 5.67 ± 1.80 cm in 67.5 kg Pupuk to 11.50 ± 3.59 cm in 87.5 kg SSI. In the 5th and 6th WAP, the height of *A. viridis* ranged from 6.33 ± 2.08 cm and 7.67 ± 2.34 cm in 67.5 kg Pupuk to 16.33 ± 4.95 cm and 19.33 ± 6.19 cm in 87.5 kg SSA. There were no significant ($p > 0.05$) differences in the influence of SS amendments on *A. viridis* height during its growing period except in the 4th WAP (Table 6).

TABLE 6
Effects of sewage sludge amendments on height (cm)

Soil Amendments	Week After Planting					
(kg N ha^{-1})	1st	2nd	3rd	4th	5th	6th
67.5 SSA	1.73 ± 0.31^{a}	4.17 ± 1.50^{ab}	5.00 ± 1.72^{ab}	5.67 ± 1.87^{a}	10.33 ± 3.15^{ab}	14.67 ± 4.70^{ab}
87.5 SSA	2.17 ± 0.58^{a}	5.33 ± 1.91^{b}	6.33 ± 2.12^{ab}	11.00 ± 3.32^{bc}	16.33 ± 4.95^{b}	19.33 ± 6.19^{b}
107.5 SSA	2.17 ± 1.44^{a}	4.33 ± 1.57^{ab}	5.83 ± 2.03^{ab}	7.67 ± 2.53^{abc}	10.67 ± 3.43^{ab}	15.00 ± 4.69^{ab}
67.5 SSI	1.43 ± 0.60^{a}	3.67 ± 1.55^{ab}	5.00 ± 1.93^{ab}	8.00 ± 2.84^{abc}	11.67 ± 4.14^{ab}	15.33 ± 5.40^{ab}
87.5 SSI	2.00 ± 0.00^{a}	5.17 ± 1.97^{b}	7.00 ± 2.40^{b}	11.50 ± 3.59^{c}	15.67 ± 4.92^{b}	18.67 ± 6.00^{b}
107.5 SSI	2.33 ± 0.58^{a}	4.17 ± 1.50^{ab}	4.50 ± 1.72^{ab}	7.00 ± 1.87^{ab}	10.00 ± 3.13^{ab}	12.67 ± 4.37^{ab}
67.5 SSO	1.50 ± 0.50^{a}	3.83 ± 1.39^{ab}	5.00 ± 1.66^{ab}	6.33 ± 2.02^{a}	10.67 ± 3.12^{ab}	13.33 ± 4.17^{ab}
87.5 SSO	2.33 ± 0.29^{a}	3.00 ± 1.68^{ab}	3.67 ± 1.94^{ab}	6.50 ± 2.45^{a}	10.33 ± 3.58^{ab}	12.67 ± 4.57^{ab}
107.5 SSO	2.00 ± 1.00^{a}	3.33 ± 1.03^{ab}	4.67 ± 1.39^{ab}	6.50 ± 2.00^{a}	10.67 ± 3.18^{ab}	13.67 ± 4.35^{ab}
NPK	2.33 ± 0.76^{a}	4.83 ± 1.65^{b}	6.00 ± 2.07^{ab}	8.33 ± 2.82^{abc}	12.33 ± 3.56^{ab}	13.00 ± 4.37^{ab}
Control	1.70 ± 0.17^{a}	3.67 ± 1.32^{ab}	4.67 ± 1.55^{ab}	6.67 ± 2.09^{a}	9.67 ± 2.91^{ab}	11.67 ± 3.64^{ab}
Pupuk	1.67 ± 0.29^{a}	2.83 ± 0.91^{a}	4.67 ± 1.46^{a}	5.67 ± 1.80^{a}	6.33 ± 2.08^{a}	7.67 ± 2.34^{a}

Superscripts with the same letters down the column were not significantly ($p < 0.05$) different (DMRT).
SS= sewage sludge; A= Abesan; I= Ikeja; and O= Oke-afa

Effects of sewage sludge amendments on biomass. Table 7 shows the effects of sewage sludge amendments on biomass. The biomass weight ranged from 3.05 ± 0.07 g in 67.5 Pupuk to 7.67 ± 0.07 g in 87.5 SSA with high significant ($p < 0.05$) difference (Table 7). The plant growth parameters assessed for phytotoxicity had similarly positive response, probably due to contribution of adequate biogenic (N and P) contents of the SS amendments as corroborated with the work of Wu *et al.* [42] that demonstrated enhanced shoot and root growth in Indian mustard on application of N and P. Similarly, Rahman *et al.* [11] revealed that N enriched fertiliser significantly improved the growth parameters. It might also be that the soil

TABLE 7
Effects of sewage sludge amendments on biomass (g)

Amendments (kg N ha^{-1})	Mean±S.D
67.5 SSA	6.41 ± 0.04^{e}
87.5 SSA	7.67 ± 0.07^{g}
107.5 SSA	6.62 ± 0.11^{e}
67.5 SSI	7.32 ± 0.01^{f}
87.5 SSI	7.37 ± 0.03^{f}
107.5 SSI	5.26 ± 0.13^{c}
67.5 SSO	5.55 ± 0.26^{d}
87.5 SSO	5.40 ± 0.06^{cd}
107.5 SSO	6.41 ± 0.15^{e}
67.5 NPK	5.56 ± 0.23^{d}
Control	4.12 ± 0.14^{b}
67.5 Pupuk	3.05 ± 0.07^{a}

Superscripts with the same letters down the column were not significantly ($p < 0.05$) different (DMRT).
SS= sewage sludge; A= Abesan; I= Ikeja; and O= Oke-afa

conditions became more stable to plant growth after a certain period when plants can produce more leaves, increase height and basal [43]. It was also observed that there were no significant ($p > 0.05$) differences in the height of *A. viridis* within the six weeks growing period, except in the 4th WAP, the growth appreciated in the order 87.5, 107.5 and 67.5 kg N ha^{-1}respectively.

The amendments at 67.5 kg N ha^{-1} performed in the order of SS > NPK > Control, thus showing the huge contributions of SS samples. This further confirmed that SS could increase yields of some crops three times more than using commercial fertilisers on sandy or clay soils [12] by providing supports for fast growth rate of the test plant [44] with no phytotoxic effects [21]. Soil amended with SS increased length of root and shoot, total chlorophyll contents [45] and plant biomass [12; 45]. Soil cation exchange capacity improved with SS application [46]. In turn, this made more adsorption sites available for essential nutrients at the root zone and limited HMs for bioavailability [47; 48]. There was no phytotoxic effect on any of the growth parameters assessed on *A. viridis* Linn similar to the past studies of Melo *et al.* [22] and Vaca *et al.* [23]. The studies revealed no phytotoxic effects on the maize growth parameters and yields probably as a result of low contents of pollutants and enriched organic matter on which the contaminants might adsorb.

Effects of sewage sludge amendments on A. viridis shoot accumulation. Safe consumption of vegetable shoot might necessitate determination of toxic metals to prevent food contamination [49]. Table 8 showed accumulation of Zn in the stems with values varying from 0.85 ± 0.13 in 87.5 kg SSA to 2.08 ± 0.14 mg kg^{-1}in 67.5 kg SSA, while the concentrations in the leaves ranged from 0.58 ± 0.10 in 87.5 kg SSA to 1.92 ± 0.08 mg kg^{-1}in 67.5 kg SSI significantly ($p < 0.05$). Other observed metals were below the detection limits in the shoots, similar to the study of Maşu *et al.* [50] who observed that inhibition of Pb and Ni transfer into aerial part of plant tissue is a way some plants tolerate and minimise phytotoxic effects. Reason might also result from the soil pH (7.78), which was close to the neutral pH (6.8 to 7.2) [51; 52]. These studies demonstrated that soil with pH close to neutral pH could cause low HMs accumulation by vegetables. Khalid *et al.* [6] observed that the most crucial factor that controls metal mobility in soil is pH, which is the most effective and rapid method of controlling the bioavailability of HMs in SS treated soil. Woodbury [53] also affirmed that soil pH influences availability of ions.

King and Dunlop [54] observed that organic matter (OM) content of SS is another factor that moderates bioavailability of HMs in soil, and an essential adsorptive medium for trace metals (Cr and Pb). Fletcher and Beckett [55] affirmed that soluble OM from SS has two groups of exchange sites binding different cations. Khalid *et al.* [6] reiterated that free metal activity in soil solution happened to reduce significantly by organic ligands, OM. Oleszczuk [56] observed that level of phytotoxicity depends on soil type, plants species and nature of SS. Processes that include adsorption, desorption, bioformation, volatilisation, photodegradation, bioaccumulation, leaching and humic substances sequester residue formation [57], control pollutants availability [58] and determine HMs exhibited phytotoxicity [59]. Comparatively, the values of HMs were lower than the values detected by Lokeshappa *et al.* [60] whose results from nine vegetable samples were higher than the permissible limits. Several studies have indi-

TABLE 8

Effects of sewage sludge amendments on *A. viridis* shoot accumulation

Amended Quantities (kg N ha^{-1})	Stem	Leaf	Shoot (stem + leaf)
67.5 SSA	2.08±0.10^{d}	1.80±0.20^{ef}	3.88 ± 0.30
87.5 SSA	0.85±0.13^{a}	0.58±0.10^{a}	1.43 ± 0.23
107.5 SSA	1.40±0.05^{b}	0.62 ±0.12^{a}	2.02 ± 0.17
67.5 SSI	1.52 ±0.08^{bc}	1.92 ±0.08^{f}	3.44 ± 16
87.5 SSI	1.60±0.10^{c}	1.27±0.08^{b}	2.87 ± 18
107.5 SSI	1.60±0.10^{c}	1.38±0.23^{bc}	2.98 ± 0.33
67.5 SSO	1.47±0.06^{bc}	1.60±0.05^{cde}	3.07 ± 11
87.5 SSO	1.43±0.08^{bc}	1.57±0.08^{cd}	3.00 ± 16
107.5 SSO	1.50±0.05^{bc}	1.63±0.10^{de}	3.13 ± 60
NPK	1.52 ±0.08^{bc}	1.67±0.15^{de}	3.19 ± 23
Control	1.35±0.18^{b}	1.55±0.05^{cd}	2.90 ± 23
Pupuk	1.43±0.08^{bc}	1.57±0.08^{cd}	3.00 ± 16

Superscripts with the same letters down the column were not significantly ($p < 0.05$) different (DMRT).
SS= sewage sludge; A= Abesan; I= Ikeja; and O= Oke-afa

cated higher values of HMs in environmental samples [61; 62; 63; 64; 65] (Table 9). No Zn values observed in the shoots (stems and leaves) for all the SS amended soil exceeded EPAC (Environmental Protection Administration, China) limits (100.00 mg kg^{-1}) for vegetables [66]. Non-detection of Cd in the samples analysed in this study was similar to Antonious *et al.* [67], where Cd was not detected in the edible part of green grown in SS amended soil but was detected in soils fortified with other amendments.

CONCLUSION AND RECOMMENDATIONS

This study has evaluated Sewage Sludge (SS) samples tested on *Amaranthus viridis* Linn for phytotoxic effects. The SS was significantly enhanced the growth and yield of *Amaranthus viridis*. It contributed to the nutrient contents of the soil. All the SS samples performed similarly as amendments and influenced the test plant better than the commercial inorganic fertilisers (NPK and Pupuk). The SS posed no phytotoxic effects on the test plant growth parameters and yield and influenced the plant to have optimum growth and yield at 87.5 kg N ha^{-1}. There was no significant difference in the height of *A. viridis* within the six weeks growing period, except in the 4^{th} WAP. The performances were in the decreasing pattern of SS > NPK > Control. All the selected heavy metals except Zn were below the detection limits in the edible parts (shoots) of vegetable. It showed that SS samples carried a low risk of heavy metal toxicity as a soil amendment. This study therefore recommends sustainable exploration of SS as amendments to create relief to waste disposal problems and further monitoring of metals in SS samples for protection of human health.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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TABLE 9

Comparison of HMs concentration (mg kg^{-1}) in this study with other studies

Cr	Zn	Cu	Cd	Pb	Authors
2.30	9.40	73.30	0.20	0.30	[68; 69]
-	2.25 - 47.5	5.03 - 24.40	-	-	[61]
-	7.30 – 34.80	5.10 – 8.30	0.11 – 0.20	-	[62]
-	264 – 1, 107	3.44 – 22.00	-	8.90 – 18.80	[63]
5.10 – 13.00	6.30 – 150.30	5.10 - 10.80	-	-	[64]
0.30	-	40.00	0.20	0.30	[70]
0.07 - 19.00	5.94 –270	1.51 - 32.10	0.01 - 0.8	0.15 – 38	[65]
BDL	1.43 - 3.88	BDL	BDL	BDL	This Study

BDL = Below detection limit

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