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Environmental Status of Discharged Hospital Wastewater and its Effects on Morpho-physiological Characteristics of *Vigna unguiculata*, *Zea mays* and *Glycine max*

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ABSTRACT

Background and Objective: There is an increasing universal awareness of environmental problems arising as a result of pollution, especially in Nigeria. Among the sources of this problem is effluent discharge from industries, particularly hospitals in arable farmlands and environs. This study was carried out to ascertain the environmental status of discharged hospital wastewater and its Effects on early seedling establishment of *Vigna unguiculata, Zea mays* and *Glycine max*. **Materials and Methods:** Discharged wastewater from the three locations (WWSP1, WWSP2 and WWSP3) was collected and homogenized for the bioassay. The concentration was prepared by mixing appropriate volumes of distilled water with the wastewater effluents to obtain 0, 3, 6, 9 and 13% (v/v). Each concentration served as a treatment. Physicochemical properties of the waste water samples were determined using established protocols while heavy metals concentrations were obtained using AAS. The microbial load and frequency of occurrences were determined using an established procedure. **Results:** There was no significant difference ($p>0.05$) in the physicochemical parameters of the wastewater samples across the three sampling sites. The heavy metals identified in the wastewater were Pb (mg/kg), As (mg/kg), Cr (mg/kg), Cd (mg/kg), Ni (mg/kg) and Hg (mg/kg) at varying concentrations ranged from 0.015±0.2 to 0.927±3.34d mg/kg. These values were within WHO permissible limits. Microorganisms isolated from the wastewater were *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis* and *Klebsiella pneumonia*. The growth parameters of the test plants were significantly ($p<0.05$) affected in a dose-dependent manner. **Conclusion:** Therefore, the study established that hospital effluent can pose environmental health risks and possibly affect early seedling establishment when not properly treated before discharge.

KEYWORDS

Hospital wastewater, microbial load, *Vigna unguiculata*, *Zea mays*, *Glycine max*, Umuahia

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INTRODUCTION

Globally, hospitals are known to play an essential role in the maintenance of the health status of a country's citizens. It has been established that hospitals contribute to health services by offering continual services to address complicated health scenarios[1]. In most developing countries, hospital activities are often associated with the generation of diverse inorganic, organic and microbial components usually released without prior treatment into the immediate environment[2]. These organic and inorganic wastes have been implicated to potentially pollute the ecosystem[3]. As opined by Placide *et al.*[4] wastes generated by hospital management have been a major source of concern to the environmental chemist asbdue to the presence of toxic contaminants that exert harmful impacts on human and aquatic species. Similarly, Hassen *et al.*[5] reported that daily wastewater generated in the hospital per bed varies from 40 to 120 L in developed countries and 2-50 L in developing countries like Nigeria[1].

Naturally, these hospital effluents can be the source of a variety of chemical, biological and physical dangers to public and environmental health[6]. This can result in the emergence of a number of pathogenic and non-pathogenic organisms that cause the outbreak of several water-related diseases that menace human life, especially in developing countries[7]. According to Fatimazahra *et al.*[8] these effluents have an ecotoxicity 5 to 15 higher than that of urban effluents; in addition to their therapeutic value, the pharmaceutical substances present in the hospital effluents can interfere with specific biological targets, which brings into question the ecotoxicological and health risks associated with their occurrence in the environment[9,10].

According to Kümmerer and Helmers[11], hospital effluent from wastewater treatment plants is commonly used as a source of irrigation in agriculture in many developing and developed countries. Before using wastewater in agriculture, it is better to evaluate the phytotoxic effects of wastewater on crops. Studies that assess the heavy metal concentration of hospital effluent and its effects on plant growth are still rare in Nigeria. Therefore, this Study was carried out to appraise the environmental status of discharged hospital wastewater and its effects on early seedling establishment of *Vigna unguiculata*, *Zea mays* and *Glycine max* in Umuahia, Southeast Nigeria.

MATERIALS AND METHODS

The study was carried out from September to November, 2023 at the Teaching and Research Laboratory of the Department of Biological Sciences, Hezekiah University Umudi, Imo Nigeria Umuahia, Abia State Nigeria. The 2 state owned (Umuduru General Hospital and St Paul's Hospital) and one private hospital (Herphertepes Hospital) were chosen for the study in umuahia, Abia State, Nigeria.

Sample collection: Water samples were collected from surface water (20-30 cm depth) using autoclaved Winkler bottles and autoclaved bottles for physical and chemical analysis[12]. Triplicate samples were collected and stored in a refrigerator. After collection, all three samples were further processed.

Heavy metal analysis: Heavy metal constituents in the wastewater samples were collected using AAS, following the method of Odjadjare and Okoh[13].

Microbial analysis: The method used by Justin *et al.*[14] was adopted in this study.

Plant seedling emergence and growth inhibition assay: The essence of this test is to assess the effect of seedling emergence and early growth of terrestrial plants on exposure to polluted soil in the ecosystem. In accordance with OECD 208 guideline[1], the test gives an idea of the sensitivity of plants during the early stage of development/establishment and provides data as to whether the pollutants inhibit or enhance the growth of the terrestrial plants under study.

Collection and preparation of seeds: Approximately 5 g of seeds used in this study were collected from the gene bank of International Institute of Tropical Agriculture, Ibadan. The accession used were *Vigna unguiculata*, *Zea mays*, *Glycine max* and *V. unguiculata*. The seed viability test was carried out by water floatation method following the method of Kaur *et al.*[3]. Discharged wastewater from the three locations (WWSP1, WWSP2 and WWSP3) was collected and homogenized for the bioassay. The concentration was prepared by mixing appropriate volumes of distilled water with wastewater effluents to obtain 0, 3, 6, 9 and 13% (v/v). Each concentration served as a treatment[15].

Experimental design: Ten viable seeds of each accession were placed in 26 mm Petri dishes lined with cotton wool and moistened with the measured concentration of spent engine oil. The growth chamber was set at 22°C with a relative humidity of approximately 58.9%. The seeds in each Petri dish were moistened with each level of contaminant and monitored for germination seedling growth for 14 days. The number of germinated seeds was recorded every morning at 9 am. The experiment was laid out in a Completely Randomized Design with three replicates each. Seeds were considered as germinated when the root reached 2 mm long[3]. Seeds that germinated from treatment were added cumulatively to obtain percentage germination.

Data collection: The reaction of the test plants to exposed contaminant at various concentrations was assessed in reference to control using phytotoxicity test parameters: Seed germination percentage and rate of germination, seedling root and shoot lengths after germination and recorded as follows[13]:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Statistical analysis: Data collected from this study was analysed using routine statistical tools, percentages, standard deviation and graphs One-way Analysis of Variance (ANOVA) and the differences were determined at 95% level of confidence.

RESULTS AND DISCUSSION

Physicochemical parameters of water samples collected from the study locations: The physicochemical parameters of wastewater samples collected from the study sites along with the corresponding Federal Ministry of Environment benchmarks are presented in Table 1. Results obtained showed that there was no significant difference ($p>0.05$) in temperature of the wastewater samples across the three sampling areas. In WWSP1, WWSP2 and WWSP3 the mean value of temperature ranged from 26.56±3.16 to 27.34±3.31°C. The highest value of temperature was obtained from WWSP3 area (27.34±3.31) while the least value (26.56±3.16) was obtained from WWSP2 axis. However, the

Table 1: Physicochemical parameters of water samples collected from the study locations

Parameter	WWSP1	WWSP2	WWSP3	FEMENV benchmark
Temperature (°C)	27.19±3.20	26.56±3.16	27.34±3.31	20-30
pH	5.67±1.78	5.35±1.43	6.49±1.52^{a}	6.50-8.50
Total chloride (mg/L)	14.33±1.75	15.26±1.82	11.45±1.93	250.00
TDS (mg/L)	26.32±2.35	38.23±3.11	31.31±3.03	500.00
Phosphate (mg/L)	0.33±0.22	0.29±0.02	0.36±0.02	5.00
DO (mg/L)	6.04±0.63	5.20±0.49	5.23±0.34	>5.00
Total hardness (ppm)	118.51±5.09	73.53±4.27^{a}	44.52±4.23	150.00
Nitrate (mg/L)	49.87±3.88	53.57±6.01	44.90±3.11	50.00
Turbidity (NTU)	16.56±2.06	28.45±2.38	27.67±2.38	150.00
Alkalinity (mg/L)	16.28±1.99	25.23±2.22	24.02±2.20	200.00
Sulphate (mg/L)	3.30±0.37	4.21±0.43	3.20±0.31	100.00
Ammonium (mg/L)	0.31±0.14	0.63±0.08	0.51±0.04	0.30

Mean along the row having different superscript alphabets differ significantly at $p\geq 0.01$ according to Duncan's multiple range test, TDS: Total dissolved solid and DO: Dissolved oxygen

Table 2: Heavy metal levels in wastewater from the study areas

Heavy metals	WHO (2006)	Control	WWSP1	WWSP2	WWSP3
Pb (mg/kg)	0.01	ND	0.334 ± 0.2^a	0.045 ± 0.1^b	0.015 ± 0.2^a
As (mg/kg)	0.01	ND	0.805 ± 0.3^a	0.761 ± 0.3^a	0.792 ± 0.3^a
Cr (mg/kg)	0.05	ND	0.023 ± 0.01^a	0.0272 ± 0.01^a	0.0173 ± 0.01^a
Cd (mg/kg)	0.01	ND	0.006 ± 0.03^a	0.002 ± 0.02^b	0.046 ± 0.03^a
Ni (mg/kg)	0.02	ND	0.032 ± 0.03^a	0.018 ± 0.03^a	0.031 ± 0.03^a
Hg (mg/kg)	0.01	ND	0.927 ± 3.34^d	0.0563 ± 3.53^b	0.0487 ± 3.42^c

Values are mean triplicate; which means having different superscripts of letters along the row differ significantly at $p<0.05$ using least significant difference and FEMENV: Federal Ministry of Environment

concentration temperature in all the study areas fell within the permissible limit of 20-30 which is the standard set by the Federal Ministry of Environment. The pH of the samples ranged from 5.35±1.43 to 6.49±1.52. There was no significant difference ($p<0.05$) in pH values of all the study locations with respect to pH. The values for pH in water were observed to be within 6.50-8.50 permissible limits. There was no significant difference ($p\geq0.01$) among the values for total chloride across the study areas. The values obtained for total chloride were within <250.00 permissible limits. There was a significant difference ($p>0.05$) among the TDS of water samples in all the study areas. However, the value of TDS in all the study areas was within 500 permissible limits set by the FMENV. Phosphate mean values varied from 0.29±0.02 to 0.36±0.02 with the highest value obtained from WWPS3 (0.36±0.02).

There was no significant difference ($p<0.05$) in the concentration of DO across the study areas. The values obtained for DO were all below >5.00 limit by the Federal Ministry of Environment. There was a significant difference (at $p\geq0.01$) in total hardness value across the study areas. The value obtained for total hardness was below 150 limits. The result of total hardness (ppm) in samples recorded the highest values at WWSP1 axis (118.51±5.09) while the least values were obtained from WWSP3 area (44.52±4.23). Mean value for total nitrate was higher at WWSP1 axis (49.87±3.88) while the least value was gotten from WWSP3 (44.90±3.11). Higher values of turbidity were recorded in WWSP2 (28.45±2.38) compared with the lowest value of 16.56±2.06 obtained at WWSP1. The turbidity values obtained in the entire study area were low as compared to FEMENV permissible limit of 150.00 set for turbidity in water. The highest alkalinity (mg/L) values were recorded in WWSP2 (25.23±2.22) while the least value was obtained from WWSP1 (16.28 ± 1.99^b). The values for sulphate, (Mg/L) in WWSP1, WWSP2 and WWSP3 ranged from 3.20±0.31 to 4.21±0.43. These values were within 100.00 set by the FEMENV in water. Ammonium (mg/L) concentration recorded in the study areas ranged from 0.31±0.14 to 0.63±0.08. However, ammonium concentrations across the study areas were above 0.30 benchmark by the FEMENV.

Results of the mean heavy metal concentrations in water from WWSP1, WWSP2 and WWSP3 are depicted in Table 2. Mean values of lead fluctuated as follows: WWSP1 (0.334±0.2), WWSP2 (0.045±0.1) and WWP3 (0.015±0.2). No value of Pb was recorded in the reference (control) sites. The results obtained for Pb were within 0.05 limit by the FEMNV. The concentration of arsenic ranged from 0.761±0.3 to 0.805±0.3 with no values detected at the control site. There was no significant difference at $p<0.05$ between mean values of arsenic at WWSP1, WWSP2 and WWSP3 points, respectively. However, the least mean value for arsenic was observed at WWSP2 (0.761±0.3). There was no significant difference at $p<0.05$ among the mean values of chromium in the study sites. Cadmium content of the water samples varied as follows: WWSP1 (0.006±0.03), WWSP2 (0.002±0.02) and 0.046±0.03 in WWSP3 axis. There was no significant difference at $p<0.05$ among the concentration of nickel across the sampling points. The highest value of Ni was obtained at WWSP1 (0.032±0.03), while the least mean value was obtained from WWSP2 area (0.018±0.03), with no values obtained from the control site. Similarly, there was no significant difference ($p<0.05$) in the mean mercury content of the samples. The highest mean value of Hg was obtained at WWSP1 site (0.927±3.34), while WWSP3 area (0.0487±3.42) recorded the least value of Hg.

The result revealed the presence of *Candida albicans* in the waste water as shown in Fig. 1.

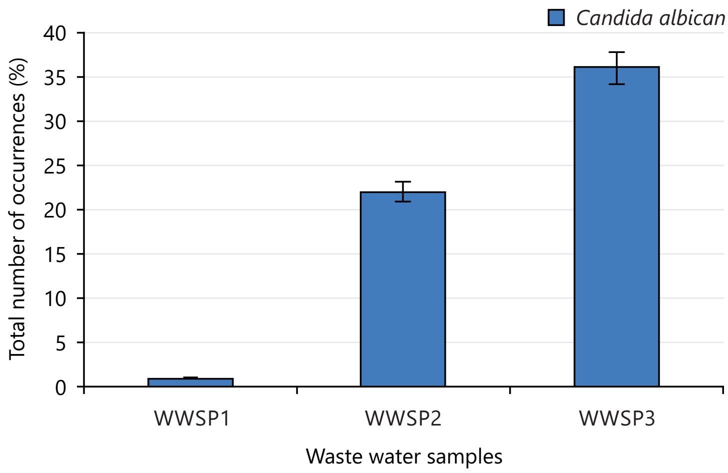

Fig. 1: Frequency of occurrences of *Candida albicans* in the hospital wastewater samples

Table 3: Mean microbial counts in the hospital wastewater samples

Mean microbial counts	WWSP1	WWSP2	WWSP3
Mean total aerobic bacteria count (cfu/mL)	$11.4\pm0.26\times10^{10}$	$10.3\pm0.15\times10^{7}$	$18.3\pm1.12\times10^{10}$
Mean total anaerobic bacteria count (cfu/mL)	$4.0\pm1.3\times10^{3}$	$3.0\pm2.3\times10^{3}$	$1.4\pm0.21\times10^{4}$
Mean total fungal count (cfu/mL)	NIL	$0.1\pm0.22\times10^{3}$	$1.4\pm0.14\times10^{5}$

Table 4: Germination of *Zea mays, Glycine max* and *Vigna unguiculata* as affected by different concentrations of hospital waste water

Concentration (v/v)	*Zea mays*	*Glycine max*	*Vigna unguiculata*
0	78 ± 1.58^{a}	71 ± 4.10^{a}	89.8 ± 2.08^{a}
3	75 ± 2.01^{a}	56 ± 3.81^{b}	39.2 ± 2.02^{a}
6	48 ± 0.31^{b}	44 ± 1.92^{ab}	25.0 ± 1.09^{b}
9	46 ± 0.10^{b}	31 ± 1.03^{c}	18.6 ± 0.21^{c}
13	23 ± 0.02^{c}	39 ± 0.88^{d}	32.9 ± 0.103^{d}

Means with different alphabets within the same column are significantly different at $p<0.05$ using the Duncan's multiple range tests and effects on early seedling establishment

Table 5: Growth parameters of *Vigna unguiculata* exposed to different concentrations of hospital wastewater

Concentration (%)	Plant height (cm)	Root length (cm)	Seedling fresh weight (kg)	Seedling dry weight (kg)
0	11.6 ± 1.16^{a}	9.4 ± 1.14^{a}	7.7 ± 1.09^{a}	6.3 ± 1.06^{a}
3	11.0 ± 1.15^{a}	8.1 ± 1.11^{a}	6.9 ± 1.03^{a}	5.8 ± 0.84^{a}
6	8.5 ± 1.13^{b}	$7.6\pm1.10^{ab`}$	5.3 ± 0.82^{b}	5.6 ± 0.71^{a}
9	7.4 ± 1.07^{b}	6.3 ± 1.06^{b}	4.9 ± 0.72^{b}	4.3 ± 0.70^{b}
13	5.7 ± 1.01^{c}	3.5 ± 0.43^{c}	3.3 ± 0.60^{c}	2.9 ± 0.44^{c}

Means with different alphabets within the same column are significantly different at $p<0.05$ using the Duncan's multiple range tests

The study also showed the presence of pathogenic and nonpathogenic organisms. The presence of these organisms may be a potential risk to nearby farms and water bodies, which may be contaminated by wastewater effluent (Fig. 2). The occurrences of the pathogenic organisms revealed in this study were in agreement with the report of Pratibha *et al.*[16]. The mean total bacteria count ranged from $18.3\pm1.12\times10^{10}$ to $10.3\pm0.15\times10^{7}$ as shown in Table 3 while that of fungal counts ranged $0.1\pm0.22\times10^{3}$ to $1.4\pm0.14\times10^{5}$ and fell within the range reported by other workers[15-18].

Effects on germination: Obtained results showed a significant difference ($p<0.05$) relationship was observed between the control, as well as raw and treated wastewater samples with respect to seed germination capacity (Table 4). According to the findings, the plant height, root length and seedling fresh and dry weights increased with an increase in the concentration of wastewater (Table 5). Implying that the hospital wastewater had a negative effect on the seed germination and early seedling establishment of the plants[7-9].

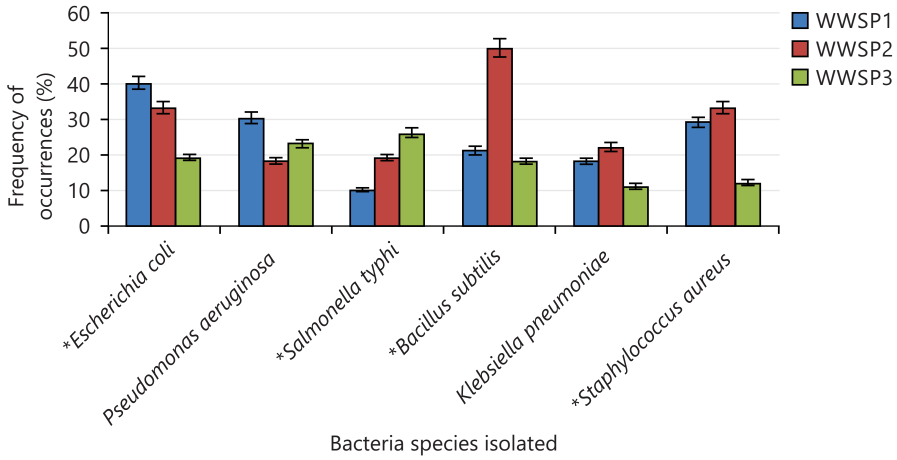

Fig. 2: Frequency of occurrences of bacteria isolates from hospital wastewater samples, pathogenic isolates are marked with (*)

CONCLUSION

The crux of this study was to evaluate the environmental status of discharged hospital wastewater and its effects on early seedling establishment of *Vigna unguiculata, Zea mays* and *Glycine max*. In Umuahia, Southeast Nigeria. The results revealed traces of heavy metals and presence of pathogenic and non-pathogenic microorganisms in the wastewater. Hospital wastewater can contain hazardous substances, such as pharmaceutical residues, chemical hazardous substances, pathogens and radioisotopes. We recommend the treatment of effluents from hospitals prior to discharge to avoid environmental pollution.

SIGNIFICANCE STATEMENT

This research aims to elucidate the impact of unregulated hospital waste disposal on the environment and the possible implications on the ecosystem. Hospital wastewater contains various potentially hazardous components which pose a potent threat to human health security concerning its high vulnerability towards the outbreak. This, in turn, leads to their dispersion and potential transmission to humans through water and the food chain. Results obtained from this study have established that indiscriminate disposal of hospital wastes alters physicochemistry of the soil which ultimately affects crop productivity. Therefore, the study calls for a critical evaluation of the current research advances, knowledge gaps and directions for future research in hospital wastewater and its removal by the existing treatment facilities.

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