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Influence of fish pond effluent on the microbiological characteristics of soil and growth of maize crop

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Abstract

Fish pond effluent is discharged untreated into the soil thereby producing objectionable odour and flies infestations. However, the effluent has been known to contain organic and inorganic nutrients and microorganisms which may promote crops growth and productivity, therefore this work studied the influence of the untreated fish pond effluent on the microbiological characteristics of soil and growth of maize crop. The microbial counts of the effluent and soil samples and growth characteristics of the maize crop were obtained using standard analytical methods. The microbial counts of the effluent-receiving soil were higher than those of the effluent samples and unpolluted soil. Bacillus subtilis (18.2%), Escherichia coli (20.5%), Serratia marcescens (13.6%), Pseudomonas fluorescens (15.9%), Lactobacillus plantarum (17.0%), Klebsiella pneumoniae (14.8%), Penicillium expansum (45.4%), Aspergillus niger (36.4%) and Aspergillus flavus (18.2%) were isolated from the effluent-receiving soil while the unpolluted soil had Staphylococcus epidermidis (28.3%), E. coli (19.6%), B. subtilis (17.4%), P. fluorescens (10.9%), Micrococcus luteus (8.7%), L. plantarum (6.5%), Kl. pneumoniae (4.3%), S. marcescens (4.3%), P. expansum (40.0%), Aspergillus niger (20.0%) and Aspergillus flavus (40.0%). The maize crop grown on the effluent-receiving soil had better growth characteristics than those planted on the unpolluted soil. This study indicated that the untreated fish pond effluent had positive effect on the soil microbial populations and enhanced the growth of maize crop, therefore, its use in agriculture to enhance soil fertility and crop growth is advocated.

Keywords: Effluent, fish, pond, maize, soil, microorganisms.

Introduction

Fish is rich in protein which is a macronutrient essential in building of the body muscle mass. A continuous dietary supply of proteins is required to supply the essential amino acids needed for the growth and maintenance of the body. The aquatic habitat provides the enabling environment for the growth and development of fishes. In Africa, fishes for commercial uses are reared in ponds made of concrete, plastic, glass and metals among other materials as a result of the unavailability of land. These fishes are fed with feeds and sometimes organic manure which may contain both pathogenic and beneficial microorganisms. Contamination of such ponds may also come from the air, fish farmers and the source water.

Up to 80% of feed ingested by fish is released to the pond environment as faecal solids and dissolved nutrients and organic matter, with just about 20% retained as fish biomass. In the system, these solids could generate additional oxygen demand, carbon (IV) oxide and ammonia nitrogen when undergoing bacterial decomposition and if released into the natural environment, can be detrimental to aquatic habitats[1]. The method of harvesting from an earthen pond can have a huge bearing on the concentration of settleable solids[1]. This and the heavy algal blooms, common in the tropics may result in the discharge of huge volume of suspended solids that will contribute to high turbidity and biochemical oxygen demand in natural systems. When fishes are recovered from ponds, the effluent is often drained presenting both an environmental challenge and agricultural opportunity[2]. The effluents are often allowed to run into natural waterways.

Effluents from fertilized ponds can have relatively high nutrient concentrations and in turn can be potential sources of pollution and eutrophication for receiving waters[2]. Fish farming is a source of livelihood for a reasonable number of people in Nigeria. These fishes are reared in ponds including the concrete type. Effluents from some of these ponds are discharged untreated as wastes on the nearby soil resulting in the production of offensive odour and insects infestations. This work therefore studied the influence of untreated fish pond effluent on the microbiological characteristics of soil and growth of maize crop with a view to determining if the waste can be converted to agricultural and hence economic gains.

Materials and methods

Study location: This study was carried out in Asaba, the capital of Delta State, Nigeria. Asaba is located between latitude 6.2059^0N and Longitude 6.6959^0E. The area has a tropical rainforest vegetation with an annual temperature of 28^0C, annual rainfall of 1950.7mm and a cycle of rainy (March-October) and dry (November-February) seasons.

Samples collection: The effluent samples were collected from a concrete fish pond located at Jesus Saves Street, Asaba, Delta State, Nigeria into sterile plastic containers while five kilograms of the soil that had been receiving the effluents for one year were collected. In addition, five kilograms of unpolluted soil from a different location were collected and served as the control. Both soil samples were collected with sterile hand trowels at a depth of ten centimeters into sterile polythene bags and conveyed to the laboratory within one hour of collection for analysis.

Microbiological analysis of the effluent sample, effluent-receiving soil and unpolluted soil: The total bacterial and total fungal counts were determined as described by Cheesbrough[3].

Determination of the total bacterial counts: The effluent sample, effluent-receiving soil and unpolluted soil were serially- diluted. Serially-diluted samples were spread-plated on autoclaved and cooled sterile nutrient agar and incubated at 30^0C for twenty-four hours after which the viable bacteria were enumerated and expressed as colony forming units per gram and millilitre for the soil and effluent samples respectively.

Determination of the total fungal counts: The effluent samples, effluent-receiving soil and unpolluted soil were serially-diluted tenfold. 0.1ml of each serially-diluted sample was inoculated on sterile Sabouraud dextrose agar using the spread plate method and was incubated at 30^0C for 72 hours. The colonies that grew were counted and recorded as colony forming units per gram for the soil samples and per millilitre for the effluent sample.

Purification of the bacterial and fungal isolates: The bacterial and fungal isolates were purified by repeated subculturing on plates of sterile nutrient agar and Sabouraud dextrose agar respectively. The plates were incubated at 30^0C for 24hours and 30^0C for 72 hours for the bacterial and fungal isolates respectively after which the purified bacterial colonies were transferred on sterile nutrient agar and the fungal isolates on SDA slants and stored at 4^0C in a refrigerator for characterization and identification studies.

Characterization and identification of the bacterial and fungal isolates: The bacterial and fungal isolates were characterized and identified as described by Cheesbrough[3]. The tests carried out were catalase test, oxidase test, indole test, voges proskauer test, sugar fermentation test, gram staining, motility test, methyl red test, coagulase test, citrate utilization test, urease test, spore test, lactophenol cotton blue staining and slide culture test.

Influence of the untreated effluent on the growth of maize crop: The maize seeds used for the study were supplied by the Ministry of Agriculture and Natural Resources, Asaba, Delta State, Nigeria and were cultivated on both the effluent-receiving soil and unpolluted soil in November,2018. The leaf length, shoot length, root length, leaf number and crop height were measured at two weeks interval for twelve weeks.

Measurement of the leaf length, shoot length, root length, leaf number and crop height: A measuring tape was used to determine the leaf length, shoot length, root length and plant height while the leaf number was counted visually.

Data analysis: Pearson's correlation was used to analyse the data obtained.

Results and discussion

The total bacterial counts of the effluent sample, effluent-impacted soil and impacted soil are shown in Table-1. The bacterial counts of the effluent sample ranged from 5.4×10^6 to 6.9×10^6cfu/ml, effluent-impacted soil, 6.2×10^6 to 7.7×10^6 cfu/g and impacted soil, 1.5×10^6 to 3.5×10^6cfu/g.

Table-1: Total bacterial counts of the effluent sample, effluent-impacted soil and impacted soil.

Sample	Effluent sample ($\times10^6$ cfu/ml)	Effluent-receiving soil ($\times10^6$ cfu/g)	Unpolluted soil ($\times10^6$ cfu/g)
1	6.1	6.5	2.2
2	5.4	6.4	1.5
3	6.0	6.7	1.7
4	5.8	6.2	2.0
5	6.2	6.9	1.8
6	5.8	6.4	2.1
7	5.9	6.8	2.9
8	5.7	6.4	1.9
9	6.3	7.0	3.5.
10	5.9	6.3	2.8
11	6.6	7.3	3.1
12	6.9	7.7	3.3

The total fungal counts of the effluent sample, effluent-receiving soil and unpolluted soil are shown in Table-2. The fungal counts of the effluent sample ranged from 1.4×10^6 to 2.5×10^6 cfu/ml; effluent-receiving soil, 1.8×10^6 to 3.1×10^6 cfu/g and unpolluted soil, 0.1×10^6 to 1.0×10^6 cfu/g.

The characteristics of the bacterial isolates from the effluent samples, effluent-receiving soil and unpolluted soil are shown in Table-3.

Table-2: Total fungal counts of the effluent sample, effluent-receiving soil and unpolluted soil.

Sample	Effluent sample ($\times 10^6$ cfu/ml)	Effluent-receiving soil ($\times 10^6$ cfu/g)	Unpolluted soil ($\times 10^6$ cfu/g)
1	2.0	2.5	0.8
2	1.9	2.4	0.5
3	2.1	2.7	0.3
4	2.3	2.6	0.6
5	2.2	2.8	0.2
6	1.9	2.4	0.3
7	1.4	1.8	0.1
8	2.0	2.3	0.8
9	1.7	2.2	0.4
10	1.8	2.4	0.3
11	2.5	3.1	0.0
12	2.2	2.9	0.9

Table-3: Characteristics of the bacterial isolates from the effluent sample, effluent-receiving soil and unpolluted soil.

Colour of Colonies	Form	Spore test	Gram staining	Catalase test	Citrate test	Oxidase test	Methyl red test	Voges proskauer	Coagulase test	Indole test	Urease test	Motility test	Sugar fermentation test						Identity
													Glucose	Sucrose	Lactose	Mannose	Arabinose	Maltose	
Slightly yellow	Rod	+	+	+	+	+	-	+	-	-	+	+	+	+	-	+	+	+	Bacillus subtilis
Greyish white	Rod	-	-	+	-	-	+	-	-	-	-	+	+	+	+	+	+	-	Escherichia coli
White	Coccus	-	+	+	+	-	+	+	+	-	+	-	+	+	+	+	-	+	Staphylococcus epidermidis
Red	Rod	-	-	+	+	-	-	+	-	-	+	+	+	+	-	+	-	+	Serratia marcescens
Greenish yellow	Rod	-	-	+	+	+	-	+	-	-	+	+	+	-	-	+	-	-	Pseudomonas fluorescens
Bright yellow	Coccus	-	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	Micrococcus luteus
Creamy White	Rod	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	Lactobacillus plantarum
Cream	Rod	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	+	+	Klebsiella pneumoniae

Key: + = Positive, - = Negative.

The bacterial isolates were Bacillus subtilis, Escherichia coli, Staphylococcus epidermidis, Serratia marcescens, Pseudomonas fluorescens, Lactobacillus plantarum, Micrococcus luteus and Klebsiella pneumoniae.

The characteristics of the fungal isolates from the effluent sample, effluent-receiving soil and unpolluted soil are presented in Table-4. The isolates were Aspergillus niger, Aspergillus flavus and Penicillium expansum.

Table-4: Characteristics of the fungi isolated from the effluent sample, effluent-receiving soil and the unpolluted soil.

Colonial characteristics	Microscopic characteristics	Identity
Blue to green colonies	Septate hyphae with conidiophores bearing brush-like conidia	Penicillium expansum
White colonies which changed to black with rapid growth	Septate and multi-nucleate hyphae with conidia.	Aspergillus niger
White colonies which changed to brown with rapid growth	Septate and multi-nucleate hyphae with conidia	Aspergillus flavus

The occurrence of the bacterial isolates in the effluent sample, effluent-receiving soil and unpolluted soil is shown in Table-5. All the isolates were detected from the unpolluted soil, while all except Staphylococcus epidermidis and Micrococcus luteus were present in the effluent-receiving soil. In addition, the effluent sample had all the bacterial isolates except Lactobacillus plantarum and Klebsiella pneumoniae.

Table-5: Occurrence of the bacterial isolates in the effluent sample, effluent-receiving soil and unpolluted soil.

Bacterial isolates	Effluent sample	Effluent - receiving soil	Unpolluted soil
Bacillus subtilis	+	+	+
Escherichia coli	+	+	+
Staphylococcus epidermidis	+	-	+
Serratia marcescens	+	+	+
Pseudomonas fluorescens	+	+	+
Micrococcus luteus	+	-	+
Lactobacillus plantarum	-	+	+
Klebsiella pneumoniae	-	+	+

+ = detected. - = not detected.

The occurrence of the fungal isolates in the effluent sample, effluent-receiving soil and unpolluted soil is presented in Table-6. All the isolates were detected in the effluent-receiving soil and unpolluted soil while all isolates except Penicillium expansum were present in the effluent sample.

Table-6: Occurrence of the fungal isolates in the effluent sample, effluent- receiving soil and unpolluted soil.

Fungal isolates	Effluent sample	Effluent-receiving soil	Unpolluted soil
Penicillium expansum	–	+	+
Aspergillus niger	+	+	+
Aspergillus flavus	+	+	+

+ = detected. - = not detected.

Table-7 showed the frequency of isolation of the bacterial isolates in the effluent sample, effluent –receiving soil and unpolluted soil. Bacillus subtilis (23.7%) occurred most frequently followed by Escherichia coli (22.0%), Staphylococcus epidermidis (20.3%), Pseudomonas fluorescens (13.6%), Micrococcus luteus (11.9%) and Serratia marcescens (8.5%) in the effluent sample. Escherichia coli (20.5%) had the highest occurrence, followed by Bacillus subtilis (18.2%), Lactobacillus plantarum (17.0%), Pseudomonas fluorescens (15.9%), Klebsiella pneumoniae (14.8%) and Serratia marcescens (13.6%) in the effluent-receiving soil while Staphylococcus epidermidis (28.3%) was isolated most frequently, followed by Escherichia coli (19.6%), Bacillus subtilis (17.4%), Pseudomonas fluorescens (10.9%), Micrococcus luteus (8.7%), Lactobacillus plantarum (6.5%), Klebsiella pneumoniae (4.3%) and Serratia marcescens (4.3%) in the unpolluted soil.

Table-7: Frequency of isolation (%) of the bacterial isolates in the effluent sample, effluent-receiving soil and unpolluted soil.

Bacterial isolates	Effluent sample	Effluent-receiving soil	Unpolluted soil
Bacillus subtilis	23.7	18.2	17.4
Escherichia coli	22.0	20.5	19.6
Staphylococcus epidermidis	20.3	0.0	28.3
Serratia marcescens	8.5	13.6	4.3
Pseudomonas fluorescens	13.6	15.9	10.9
Micrococcus luteus	11.9	0.0	8.7
Lactobacillus plantarum	0.0	17.0	6.5
Klebsiella pneumoniae	0.0	14.8	4.3

The frequency of isolation of the fungal isolates in the effluent sample, effluent-receiving soil and unpolluted soil are shown in Table-8. Aspergillus flavus (53.3%) and Aspergillus niger (46.7%) occurred in the effluent sample while Penicillium expansum (45.4%) had the highest frequency, followed by

Aspergillus niger (36.4%) and Aspergillus flavus (18.2%) in the effluent-receiving soil. The unpolluted soil had Penicillium expansum (40.0%), Aspergillus flavus (40.0%), Aspergillus niger (20.0%).

Table-8: Frequency of isolation (%) of the fungal isolates in the effluent sample, effluent- receiving soil and unpolluted soil.

Fungal isolates	Effluent sample	Effluent-receiving soil	Unpolluted soil
Penicillium expansum	0.0	45.4	40.0
Aspergillus niger	46.7	36.4	20.0
Aspergillus flavus	53.3	18.2	40.0

The growth characteristics of the maize crop grown on the effluent-receiving soil are shown in Table-9. The mean leaf length ranged from 9.00+0.10 to 58.20+0.00cm, mean shoot length from 12.60+0.00 to 63.00+0.10cm, mean root length from 6.00+0.10 to 17.20+0.00cm, mean leaf number from 5+0.00 to 12+0.00cm and mean crop height from 10.00+0.10 to 27.20 +0.02cm from 2 to 12weeks of the maize crop growth.

The growth characteristics of the maize crop grown on the unpolluted soil are presented in Table-10. The mean leaf length ranged from 5.56±0.20 to 38.21±0.10cm, mean shoot length from 7.50±0.00 to 54.00±0.01cm, mean root length from 3.30±0.10 to 12.30±0.00cm, mean leaf number from 4±0.00 to 10±0.01cm while the mean crop height ranged from 8.10±0.00 to 22.12±0.10cm from two to twelve weeks of the growth of the maize crop.

Table-9: Growth characteristics of maize crop grown on the effluent- receiving soil.

Period of Growth (Weeks)	Leaf Length (cm)	Shoot Length (cm)	Root Length (cm)	Leaf number	Crop height (cm)
2	9.00±0.10	12.60±0.00	6.00±0.10	5±0.00	10.00±0.10
4	15.10±0.13	20.01±0.02	10.00±0.00	8±0.00	14.50±0.02
6	25.50±0.01	36.10±0.00	14.50±0.01	9±0.01	19.40±0.01
8	36.00±0.00	48.20±0.10	16.20±0.00	10±0.00	23.10±0.11
10	42.11±0.12	60.05±0.01	17.10±0.10	11±0.02	24.60±0.01
12	58.20±0.00	63.00±0.10	17.20±0.00	12±0.00	27.20±0.02

Table-10: Growth characteristics of maize crop grown on the unpolluted soil.

Period of Growth (Weeks)	Leaf Length (cm)	Shoot Length (cm)	Root Length (cm)	Number of Leaves	Plant height (cm)
2	5.56±0.20	7.50±0.00	3.30±0.10	4±0.00	8.10±0.00
4	11.20±0.01	16.10±0.20	5.50±0.00	6±0.20	12.00±0.10
6	20.60±0.10	30.50±0.01	6.20±0.01	7±0.10	17.20±0.12
8	30.12±0.13	40.11±0.20	10.50±0.02	8±0.00	19.10±0.10
10	34.14±0.02	51.20±0.10	11.30±0.10	9±0.10	20.20±0.00
12	38.21±0.10	54.00±0.01	12.30±0.00	10±0.01	22.12±0.10

Discussion: The highest bacterial counts (Table-1) were observed in the effluent- receiving soil ($6.2x10^6$ – $7.7x10^6$ cfu/g), followed by the effluent samples ($5.4x10^6$ – $6.9x10^6$ cfu/ml) and finally the unpolluted soil ($1.5x10^6$ – $3.5x10^6$ cfu/g). The fungal counts (Table-2) of the effluent-receiving soil ($1.8x10^6$ – $3.1x10^6$ cfu/g) was also higher that the counts of the effluent samples ($1.4x10^6$ – $2.5x10^6$ cfu/ml) and the unpolluted soil ($0.1x10^6$ – $1.0x10^6$ cfu/g). Eze and Ogbaran[4] and Njoku et al.[1] reported that ponds contain microorganisms and chemicals that can cause disease to the fish and eventually to man when consumed.

The higher counts obtained in the effluent-receiving soil compared with the effluent samples and unpolluted soil might be attributed to the nutrient status of both the pond and the effluent- receiving soil.

Eight bacterial isolates were recovered from the effluent samples, effluent-receiving soil and unpolluted soil. They were Bacillus subtilis, Escherichia coli, Staphylococcus epidermidis, Serratia marcescens, Pseudomonas fluorescens, Micrococcus luteus, Lactobacillus plantarum and Klebsiella pneumoniae (Table-3). These bacteria were also isolated by Kathleen et al.[6] from aquaculture in Borneo, Hindawi, Malaysia, Oni et al.[7] isolated Pseudomonas aeruginosa, Pseudomonas sp, Aeromonas hydrophila, Shigella sp, Proteus vulgaris and Staphylococcus aureus from water column as well as the parts of Clarias gariepinus collected from a fish farm in the Ile Ife. Douglas and Isor[8] reported the frequency of occurrence of Escherichia coli (17.0%) Salmonella (14.5%), Micrococcus (5.8%), Bacillus (9.4%), Staphylococcus (13.0%), Pseudomonas (8.7%) Shigella (7.3%), Klebsiella (5.8%), Streptococcus (4.4%), Proteus (3%) and Enterobacter (11%) in pond water from Ogoniland, Nigeria.

Penicillium expansum, Aspergillus niger and Aspergillus flavus were isolated from the effluent samples, effluent-receiving soil and unpolluted soil (Table-4). The result agreed with that of Njoku *et al.*[5] who isolated Aspergillus sp, Penicillium sp, Cladosporium sp, Mucor sp and Fusarium sp from some fish pond water within the Niger Delta Region of Nigeria and Obire and Anyanwu[9] who isolated the genera Alternaria, Rhodotorula, Aspergillus, Candida, Cephalosporium, Cladosporium, Fusarium, Geotrichum, Mucor, Penicillium, Rhizopus, Saccharomyces, Torulopsis and Trichoderma from soils. Iqbal and Saleemi[10] reported the isolation of Aspergillus spp (78.5%), Blastomyces sp (7.5%), Penicillium sp (3.5%) and unidentified fungal hyphae (10.5%) from a freshwater commercial fish, Catla catla. The microbial isolates had varied occurrence and frequency of isolation in the effluent samples, effluent-receiving soil and unpolluted soil (Tables-5, 8).

The maize crop cultivated on the effluent-receiving soil (Table-9) had higher mean leaf length, mean shoot length, mean root length, mean leaf number and mean crop height than the crop cultivated on the unpolluted soil (Table-10) indicating that the effluent contributed significant level of nutrients to the crop cultivated on the effluent-receiving soil. Osaigbovo and Orhue[11] reported that nutrient uptake, collar girth and leaf area were enhanced while the plant height as well as the number of leaves were depressed compared to the control. The chlorophyll content was also enhanced at low concentration of pharmaceutical effluent. There was a strong correlation between the effluent-receiving soil and the bacterial and fungal counts.

Bacillus subtilis is known to promote plant growth by inducing systemic resistance, antibiosis and competitive omission, solubilizing soil phosphorous, enhancing nitrogen fixation and producing siderophores that suppress the growth of pathogens[12]. Environmental E.coli is a natural growth promoting soil bacterium. Chandra et al.[13] reported that the inoculation of maize grains with E.coli (NBRIAR3) enhanced the plant growth and nutrient uptake and recommended that E.coli should be recognized as a native soil bacterium instead of as an indicator of the possible presence of other faecal coliform bacteria.

Staphylococcus epidermidis has been shown to possess hydrolytic enzyme activity, siderophores production and volatiles synthesis for inhibiting the plant pathogenic fungi Rhizoctonia solani which positively affects plant developments[14,15].

Studies have reported the potentials of Serratia marcescens to induce plant growth by enhancing the production of phytohormones and solubilizing phosphate[16]. A study carried out by Khaiden and Guari[17] showed that Serratia marcescens significantly improved shoot length by 95.52%, fresh shoot weight by 602.38%, fresh root weight by 438% and area of leaves by 127.2%.

Pseudomonas fluorescens has been reported to possess properties including nitrogen accumulation, siderophores production and promotion of solubilization of phosphate, silicate and zinc in plate assay[18]. Micrococcus luteus is known to enhance shoot and root length, plant height, total contents of chlorophylls a and b and carotenoids by producing plant growth promoting substances such as cytokine and hydrogen cyanide[19]. Lactobacillus plantarum has been reported to be an effective biofertilizer, biocontrol agent and biostimulant that can enhance nutrient availability from compost and other materials, plant growth or seed germination and alleviate various abiotic stresses[20].

Klebsiella pneumoniae is a strain of bacteria that promotes plant growth by fixing nitrogen, producing indole -3- acetic acid (IAA), gibberellic acid, siderophores and solubilizing phosphate[21-23]. Penicillin expansum is a biofertilizer and biocontrol agent and can enhance plant growth and survival against salt stress and Fusarium infection[24,25] . Aspergillus niger is a good biocontrol agent against plant parasitic nematodes through the stimulation of the immunity/defence of the plant host thereby enhancing the yield of plants[26] while Aspergillus flavus has been found to enhance plant growth under heat stress

conditions by secreting IAA, phenol and flavonoids that promote the growth of host plant species[27].

Conclusion

The fish pond effluent impacted positively on the microbiological properties of the effluent-receiving soil as the microbial populations increased significantly when compared with the unpolluted soil. The maize crop cultivated on the effluent-receiving soil also had better growth characteristics than the crop cultivated on the unpolluted soil. The higher populations of these growth- promoting organisms in the effluent-receiving soil must have enhanced the growth of the maize crop, therefore the application of fish pond effluent to enhance soil fertility and crop yield is feasible.

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