Effects of Snail Farming on Soil Microbial Spectrum and Physicochemical Properties.

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Abstract: The study was undertaken to determine the effect of snail farming on soil microbial spectrum and physicochemical properties. This was investigated using standard chemical analytical procedures and cultural techniques. Snail soil samples were collected from two locations within Owerri, including, Nekede and Irete. Snail species reared in the farms were *Achatina achatina* and A*rchachatina marginata*. The pH changed from neutral to alkaline. The temperature also increased slightly. Phosphate, sulphate, nitrate and total organic matter showed similar increase in the snail farm soil samples than in the test control sample with the differences being statistically significant. (P = 0.05).Sodium and potassium ions increased slightly while calcium and magnesium ions increased more. Thirteen bacterial species isolated from the snail farm soil samples were B*acillus*, *Pseudomonas*, *Aeromonas*, *Staphylococcus, Shigella* and *Flavobacteria, Nitrobacter*, *Escherichia coli* and *Klebsiella*, *Proteus*, *Enterobacter*, *Micrococcus* and *Salmonella species*. The most prevalent was *Bacillus* species which had 100% occurrence. The total heterotrophic bacteria (THB) had the highest counts in both snail farm soil samples and controls. Further bacteriological analysis of the snail shell bacterial loads showed the same trend as in the soil analysis. The values of the enzymatic activities estimated in both snail farm and control soil samples showed that dehydrogenase had the highest activities. These changes are attributable to the contents of the wastes and their metabolism by microorganisms. The snail wastes could serve as increased additional source of micro-organisms to the soil while the left-over food items served as added nutrient for microbial growth. Most metabolic reactions are exothermic and the cumulative effect was the slight increase in soil temperature observed in this study. Similarly, the breakdown of the protein content of the wastes caused the release of ammonia which dissolved in the soil moisture to cause the increased pH values observed.

Keywords: Snail farming, Microbial Spectrum, Physicochemical Properties, Soil.

Introduction

In times of economic problems as we are going through in Nigeria at present, interest in self-sufficiency increases. Snails being small noiseless and easy to handle animals can be reared in an urban situation without infringing on the peace of neighbours (Akinnusi, 2004 and Bryant,1994). Only a small space is needed for raising snails and they adapt themselves to a variety of conditions, hence they can be raised successfully in many cities, in small towns and on farms and villages (Agbogidi and Okonta, 2011; Okon *et al*., 2013). The amount of capital needed to start a snailry is reasonable, they can be fed with natural resources like plant leaves (Alikwe *et al*. 2013), caring for them does not involve strenuous physical exertion, so the work can be carried out with much satisfaction by physically able and disabled people, the exercise can equally be useful for occupational therapy (Ogogo *et al*. 2011 and Akinlade, 1994). Consequently, man has to look for a cheaper source of animal protein and that is where snailry comes in. (Olaniya 2004).

Snails are very important to our health because of their high protein content. (Sridhar *et al*. 2012) They are excellent antidote for hypertension, promotefertility, and are good for curing stomach disorders. The slime of snails is used for wound treatment and stop ping haemorrhage. Pregnant women are advised to take snails for its nutritional value (Adegoke *et al*. 2010)The shells can be used in feed formulation and decoration purposes (Cheney *et al*. 2004) .

Snails carry protozoa and many species of salmonella (Ebenso *et al*. 2012). They are subject to "Red leg" disease caused by bacteria in the aquatic environment that can also infect humans under certain conditions, therefore normal health practices should be used when raising them (Partapani *et al*. 2014 and Ekundayo, 2005).The raising of snails on any particular piece of land has the potential of altering the physicochemical and microbial dynamics of the snail farm soil. (Nyoagde *et al*. (2016). This is because the snails discharge their wastes within their habitat (Ibom *et al* 2012 and Dupont-Nivet *et al*., 2000) These snail wastes are organic, biodegradable and therefore alter the enzymatic activities of the affected soil. A lot of work have been done on the effects of organic wastes on soil physicochemical and microbial quality but records show that work on snailry are very scanty where they exist (Ekundayo,2005).

This study was therefore designed to assess the effect of snail rearing on some soil physicochemical and biological indices. To achieve this aim, the study's specific objectives are to:

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1. determine the physical parameters of the snailry impacted soil.
2. determine some chemical (nutrients) quality of the soil.
3. investigate the microbial dynamics of the soil.
4. determine the enzymatic activities of the snailry soil.

Materials and Methods

The study area were snail farms located in Irete and Nekede in Owerri West LGA, Imo State.

Sample Collection

Soil Samples

The soil samples (soil mixed with snail faecal matter and control soil) and

10 snail shells washed with distilled water were collected with sterile specimen bottles. The periodicity of sampling or sample collection was twice every month from April to August. Samples collected were taken to Abia State University Uturu Laboratory within 2-4 hours for analysis. Sampling was done ten (10) times in two weeks interval for 5 months for microbiological analysis, while four (4) of such samples were analyzed for physicochemical properties.

Analysis of the Soil Bioload

The prevalence of various bacterial species in the soil was determined by the use of various culture media using the spread plate technique according to Cheesbrough (2003). The inoculation of each prepared medium was done after 10 fold serial dilution.

Determination of Microbial Population (Bioload)

From each of the 10 fold diluted samples, 0.2ml was inoculated onto Nutrient agar (NA), Sabouraud dextrose agar (SDA), MacConkey agar (MCA) and Mineral salt agar (MSA) prepared according to manufacturers' instructions for total viable counts (Cheesbrough 2003)

Characterization and Identification of Microbial Isolates

Microscopic examination, Biochemical tests and Gram staining were carried out according to Cheesbrough (2003), and Cappucino and Cherman, (1981).

Physicochemical Parameters

Physicochemical properties such as temperature, pH, phosphate (PO_4), sulphate (SO_4), and nitrate (NO_3) of the two snail farm soil samples and control soil samples were determined as stated below:

The soil temperature was determined in-situ at the site of collection of samples with mercury-in-glass thermometer (Cheesbrough (2003). The soil pH was determined ex-situ by the use of HANA 1990 model H19835 multipurpose tester. The phosphate(PO_4^{2-}), sulphate(SO_4^{2-}), and nitrate(NO_3^-) were determined according to UNEP (2004)

Furthermore the biological loads (biolaods) of some microbial groups were assessed to determine the Microbiological quality of the soil samples. These groups were total heterotrophic bacteria (THB), coliform bacteria (CB), nitrifying bacteria (NB) and phosphate solubilizing bacteria (PSB).Nutrient agar was used for THB, MacConkey agar for CB, Sabouraud dextrose agar NB,and Phosphate growth medium for PSB, using spread plate technique after 10 fold serial dilution as described by Cheesbrough (2005).

The soil total organic matter or organic carbon (TOM) was determined according to Osuji and Adesiyan, (2005), as described by Akubugwo *et al*, (2007.)

Soil enzymatic activities were determined. The soil dehydrogenase activity was determined with the method described by Cassida *et al*, (1964) as modified by Li *et al*, (2005) . Also Acid Alkaline phosphotase activities were determined according to Tabatabai and Bremner (1969).

Data Analysis

Statistical analysis was done using analysis for variance (ANOVA). Means were compared for significance using the Duncan's Multiple Range Test ($P < 0.05$).

Results

In this study, thirteen (13) bacterial species were isolated (Table1). Among the organisms observed in both farms, the most prevalent was *Bacillus* species which had 100% occurrence. *Salmonella*, *Shigella, Flavobacteria, Klebsiella* and *Proteus* species, which had low prevalence (+) in the snail waste impacted soil were not observed in the control soil samples in the farms (Table 1).

The bacterialogical loads of all the bacteria groups were higher in the Irete farm soil than the Nekede farm soil. Statistical analysis showed that the values of total Heterotrophic bacteria are statistically higher than coliform bacteria and phosphate solubilizing bacteria, the nitrifying bacteria was the lowest (Table 2). Further bacteriological analysis of the snail shell showed the same trend as in the soil analysis (Table 3). The results of the physicochemical parameters analysis of various soil samples are shown in Table 4 The pH of the two soil samples was neutral to alkaline (7.1 to 8.2) for Nekede soil sample and (7.3 to 8.7) for Irete soil sample. The temperature for Nekede soil sample and the Irete soil sample were slightly higher than that of the control soil samples. Results obtained in the analysis of phosphate, sulphate and nitrate showed similar increase in the snail farm soil samples with each parameter being statistically significant (p=0.05)(Table 4).The total organic matter values like the other values also increased significantly (p=0.05). In metallic ion concentration analysis, the values for the snail farm at Irete farm had non- statistically higher values than the other soil samples .The results of sodium and potassium ions had

non-statistical different (p=0.05) between the snail farm soil samples and the control, while the values of calcium and magnesium were very significant (p=0.05) (Table 5). The values of the enzyme activities estimated in both the snail farm and control soil samples are shown in Table 6. Of the three enzymes with activities estimated , the dehydrogenase had the highest activities with an activity range of 24.72-39.09 TPF mg^{-1} $6h^{-1}$ (Table 6). This change was statistically significant at both snail farms (p=0.05). The activities of acid phosphatase in both farms decreased while that of alkaline phosphatase increased. (Table 6). However, the activity values of both acid and alkaline phosphatase were non-statistically higher in the Irete snail farm.(p=0.05) (Table 6).

***Table1*: Occurrence Status of Various bacterial Isolates from the Snail Farms**

ORGANISMS	NEKEDE TEST	CONTROL	IRETE TEST	CONTROL
*Bacillus S*pecies	+++	+++	+++	+++
Pseudomonas Species	++	+	++	+
Aeromonas Species	++	++	++	+
Staphylococcus Species	+++	++	+++	+
Salmonella Species	+	-	+	-
Shigella species	+	-	+	-
*Flavobacteria*Species	+	-	+	-
Nitrobacter Species	++	+	++	+
Escherichia Coli Species	+++	++	+++	+
*Klebsiella*Species	++	-	++	-
Proteus Species	++	-	++	-
Enterobacter Species	+++	+	++	+
Micrococcus Species	++	+	++	+

Keys:
+=high level of occurrence
++ = higher level of occurrence
+++ =highest occurrence.

***Table2*: Snail Farm Soil Bacteria Bioload (cfu/g)**

BACTERIAL GROUPS	NEKEDE TEST	CONTROL	IRETE TEST	CONTROL
THB	6.7×10^7	5.7×10^6	7.8×10^7	5.8×10^6
CB	4.8×10^5	2.2×10^4	4.9×10^5	2.5×10^4
NB	2.1×10^4	1.4×10^3	2.8×10^4	2.4×10^3
PSB	3.4×10^4	2.4×10^3	3.7×10^4	2.7×10^3

Key:

THB	=	Total Heterotrophic bacteria
CB	=	Coliform Bacteria
NB	=	Nitrifying Bacteria
PSB	=	Phosphate solubilizing Bacteria

***Table3*: Bioloads of Snail Shell (CFU/g)**

BACTERIAL GROUPS	NEKEDE TEST	IRETE TEST
THB	4.3×10^4	4.6×10^4
CB	2.7×10^2	$2.2. \times 10^2$
NB	1.1×10^1	1.3×10^1
PSB	2.3×10^2	2.6×10^2

Key:

THB = Total Heterotrophic bacteria
CB = Coliform Bacteria
NB = Nitrifying Bacteria
PSB = Phosphate solubilizing Bacteria

***Table 4:* Physicochemical Parameters of Soil Analysis**

TEST PARAMETERS	NEKEDE TEST	CONTROL	IRETE TEST	CONTROL
pH	8.2	7.1	8.9	7.3
Temperature (^oC)	30.1	29.3	29.7	29.2
Phosphate (PO_4)	10.76	6.73	12.68	7.54
Nitrate (NO_3)	2.01	1.43	2.47	1.26
Sulphate (SO_4)	31.32	27.62	33.47	24.17
Total Organic Matter (TOM)	15.3	11.27	18.74	10.87

***Table 5*: Metallic Ion analysis of Soil Samples (mg/g)**

METAL	NEKEDE TEST SAMPLE	CONTROL	IRETE TEST SAMPLE	CONTROL
Magnesium (Mg)	3.60	1.97	3.2	1.66
Calcium (Ca)	3.72	2.11	3.81	2.01
Potassium (K)	0.69	0.61	0.77	0.63
Sodium (Na)	0.84	0.82	0.87	0.83

***Table 6*: Enzymatic Activities on the Snail Farm Soil**

ENZYMES	NEKEDE TEST	CONTROL	IRETE TEST	CONTROL
Dehydrogenase	39.09	27.26	39.92	24.72
Alkaline Phosphatase	4.32	3.42	4.67	3.39
Acid Phosphatase	3.92	4.36	3.87	4.34

Units of Measurement:

Dehydrogenase: - TPF $Mg^{-1}6h^{-1}$
Alkaline Phosphatase
Acid Phosphatase $Mg^{-1}3h^{-1}$

Discussion and Conclusion

Results of the physicochemical parameters analysis of snail farm soil samples showed increase in temperature and increased alkaline conditions. In the same vain, NO_3, SO_4 and PO_4 contents also increased in the snail wastes impacted soil above the values of the control soil samples. These changes are attributable to the contents of the wastes and their metabolism by microorganisms. Most metabolic reactions are exothermic and the cumulative effect could have caused the slight increase in soil temperature observed in this study. Similarly, the breakdown of the protein content of the wastes caused the release of ammonia which dissolved in the soil moisture to cause the increased pH values observed. A similar observation had been reported by Adesemoye, *et al.,* (2006) and Ezeronnye and Ubawa, (2005). Nwaugo *et al.,* (2008a) reported that adult *Bulinus* snails which feed on cabbages and lettuce produced wastes with tangible SO_4, NO_3 and PO_4content. Mantellin and Touraine, (2004) had

equally stated that PO_4^{2-}, $NO_3^-$and SO_4^{2-} are important components of plant tissue hence their presence in wastes of animals that feed on them could be expected. This therefore accounted for the increased values observed in the snail waste impacted soil. The increase in the total organic matter (TOM) observed in the work is a direct consequence of the snail wastes. The feacal matter of all organisms including snails is high in organic matter (Olaniya, 2004). Observations on the metallic ion concentration showed high calcium and magnesium contents in the soil. Calcium and potassium are very important components of snail feed. Sources of calcium are even added as a feed supplement in snail farming due to its significance in snail shell and egg shell formation (Olaniya, 2004). The left over feed items, and degraded egg shells could cause the increase in calcium and magnesium concentrations observed in this work. The slight increase in sodium (Na) and potassium (K) could be attributed to components of the snail feed and wastes.

The isolation of bacterial species from the soil showed that more micro-organisms were observed in the snail farm soil than the control soil. Of the 13 (thirteen) bacterial species observed in this study, five (5) were absent in the control soil samples unlike the snail farm soil samples which had all the observed organisms. This observation agrees well with Parham *et al*., (2003) and Reverodo and Melo,(2007). The increased microbial prevalence in the snail farm soil samples indicate the increased microbial nutrients and conditions of bacterial growth. The snail wastes could serve as increased additional source of micro-organisms to the soil while the left-over food items served as added nutrient for microbial growth. However, the high prevalence of soil organisms observed in this work agrees with Olaniya, (2004). This therefore accounted for the increased values observed in the snail waste impacted soil and the increase in the total organic matter (TOM) observed. Willey *et al*., (2011), also referred to the soil as home of all organisms. The use of bacterial dynamics in soil characterization has been extensively applied in various studies. The prevalence and population of the various bacterial groups could be used to assess soil fertility. Kazeev (2006) reported that many fertile soils have high bacteria counts especially the nitrifying bacteria and total heterotrophic bacteria. The increased bacterial counts of various groups observed in this work showed that snail farm wastes caused the increase of all bacterial groups studied. In the group analysis, the total heterotrophic bacteria (THB) had the highest counts. This situation had been reported extensively by Nwaugo *et al*., (2007); Onyeagba *et al.,* (2008) and Willey *et al*., (2011). Nwaugo *et al*.,(2008b), and Kazeev (2006) showed that nitrifying bacteria are very sensitive to changes in soil and are generally low in prevalence in the soil. Generally, the Coliforms are intestinal organisms and their numbers increase in soil impacted with any form of feacal matter. Adesemoye *et al*., (2006) and Nair *et al*.,(1998) agree that animal wastes are high in Coliforms and this case is no exception. The snail wastes also include feacal matters and most Coliforms survive in soil and proliferate well too.

The analysis of snail shell bacterial load showed the same trends as observed in the soil bacteriological load analysis. The observation indicated that the habitat of the snails influenced their surface contamination. Observations in soil bacteriological loads and soil pH influenced the soil enzymatic activities. Dehydrogenase is produced by all bacterial species and it is an endo-cellular enzyme whose activity is tied to the population dynamics in any given habitat (Tabatabai, 1997; Li, *et al*., 2005; Nannipieri, *et al*., 1990). Since higher bacterial loads were found in the snail farm soil, higher dehydrogenase activity was observed in the snail soil than the control soil samples.

Acid phosphatase activities decreased, alkaline phosphatase activities increased, following changes in the pH values reported. Acid phosphatase prefer acidic conditions hence, was adversely affected by the change of the control soil from neutral to alkaline (snail farm soil). On the other hand, activities of alkaline phosphatase increased as the condition became more alkaline following snail farm activities. This observation has been reported by Nwaugo, *et al*., (2008c). Observation in all the parameters investigated in this study showed non-significantly higher values in the Irete (10m x 20m) than in the Nekede snail farm (50m x50m). This observation agrees with time and accumulation of waste. The Irete snail farm has been long in existence and higher density of snails per square metre .The Irete farm, being a farm, maximum use of space was made resulting in higher amount of snail farm waste per square metre. This therefore influenced the results obtained in the physicochemical, bacterial dynamics and enzyme activities.

In conclusion, the snail farm soil samples were affected by the snail farm activities which caused higher mineral contents and bacterial populations. This resulted in higher dehydrogenase and phosphatase activities in the impacted soil.

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