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RESEARCH ARTICLE

PHYSICO-CHEMICAL CHARACTERIZATION OF BAT GUANO FROM THE VILLAGE OF MAGARAWA IN NIGER

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ABSTRACT

Bat guano is widely used in oasis basins in the Zinder region. However, no study has been conducted to determine its chemical components of this product. The objective of this present study is the physicochemical characterization of Magarawa's guano. Bat guano samples were collected from the caves of Magarawa mound and submitted for different analysis. After preparation and packaging, the samples were observed under a microscope (x10) then subjected to chemical analyzes of elements such as nitrogen, phosphorus, potassium, bases and also lipids, proteins and carbohydrates. It emerges from this study that bat guano from Magarawa occurs in small sticks 2 mm in length and very friable in the dry state with the presence of fragments of insect remains and skeleton of bats or snakes or birds. It has a high nitrogen content (10%) and low phosphorus and potassium concentrations. The concentration of dry matter and protein remains high 92.5 and 60.66% respectively. Indeed, the physico-chemical characteristics of Magarawa's guano show that this product is a good quality organic fertilizer that is easy to handle and above all competitive compared to commercial guanos.

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INTRODUCTION

Bats are ubiquitous and play a vital role in ecological balance also in nutrient cycling and forest redistribution (Zapata, 1995). Bat guano is widely used as a natural fertilizer due to its high nitrogen content and nematicidal effects (Keleher and Sara, 1996; Gomero, 1991). Bat guano stimulates the growth of soil microfauna due to its richness in essential nutrients and organic matter (Sridhar, 2006). However, with the advent of chemical fertilizers which have become the main nutritional source of plants, the use of bat guano has been ignored. Indeed, the use of chemical fertilizers and pesticides in modern agriculture has certainly improved food productivity but has also deteriorated soil conditions, the environment and human health. However, organic farming is making a comeback and gaining in importance. Although bat guano has long been used in the Zinder region as an organic fertilizer, it has never been the subject of scientific study in Niger. Thus, this study describes the physical and chemical composition of bat guano from the village of Magarawa in Niger.

MATERIAL AND METHODS

Sampling of bat guano: Samples of bat guano were collected in the caves of Magarawa hill located 27 km from the local governement of Gouré in the Zinder region of Niger. Its geographic coordinate is longitude 10°08'26"East and latitude 13°53'22" North as shown in Figure 1. In total, 5 composite samples were collected from different caves on Magarawa Hill. They were first dried at room temperature to stop the decomposition processes; then, they are put into plastic bags; finally, they are labeled according to the principles of sampling.

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Preparation of bat guano samples: The bat guano samples collected in the field were transported to the Soil Science laboratory of the Faculty of Agronomy of Abdou Moumouni University of Niamey in Niger; where they were immediately dried again (25-30 ° C). After drying, the bat guano samples were sieved using a 2 mm mesh sieve to separate the fine part from the coarse elements, in particular the stony.

Physical analyzes: The physical analyzes consisted in observing with a magnifying glass (x10) the shapes, the constituent particles and the coloring of the samples of the guano of the Magarawa bat.

Determination of humidity: The humidity level was determined according to the official AOAC method (1990). In fact, the crucibles were previously dried in an oven at 103 ° C for 30 minutes and cooled in a desiccator. They were weighed (P_0) and then 2g of the sample (P_E) were introduced. The crucibles were placed in an oven at 103 ° C for three hours. They were reweighed until a constant weight was obtained.

The following formula is used to determine the humidity level:

$$\% \text{ humidity} = \frac{P_E - (P_f - P_0)}{P_E} \times 100$$

With: P_E *= test portion dried at laboratory temperature (2 g);*
P_o *= empty weight of the crucible;*
P_f *= final weight (crucibles + oven-dried PE).*

Determination of dry matter: The dry matter was deduced by subtracting the water content:

% DM = 100 -% in water

With: *MS: dry matter*

Mineral composition of fecal matter

Incineration is carried out so as to obtain all of the cations (excluding ammonium) in the form of carbonates and other anhydrous mineral salts (AOAC, 1990). Crucibles are dried in an oven at 103 ° C for 30 minutes and cooled in a desiccator and then weighed (P_0). Next, 3 g (P_E) of the sample are introduced into these crucibles and brought to incineration in an oven at 550 ° C for 4 hours. At the end of the incineration, the crucibles are removed and cooled in a desiccator for 30 minutes before being weighed (P_f). The percentage of ash is given by the following relation:

$$\% \text{ Ash} = \frac{P_f - P_0}{P_E} \times 100$$

P_E*: Test taking;*
P_f*: Final weight (crucible + sample);*
P_0*: Empty weight of crucibles*

Guano fat content: The fat content is determined by the soxhlet extraction method using hexane as reflux solvent (AOAC, 1990). The flask is first washed and dried. The empty weight of the balloon (P0) is noted. Five grams (PE) of the sample are introduced into the extraction cartridges; then the balloon is stoppered with cotton and placed in the soxhlet. The flask is filled with approximately 300 ml of hexane and then connected to the soxhlet. The latter is connected to a refrigeration system and is connected to a cryostat making it possible to condense the vapors of the solvent intended to entrain the lipids. Extractions last 4 hours. The hexane is collected through a tap. The flask is dried in an oven at 105 ° C and cooled in a desiccator for 30 min and then weighed. The fat content is obtained according to the following formula:

$$\% \text{ fat} = \frac{P_f - P_0}{P_E} \times 100$$

P_E*: Test sample;*
P_f*: mass of the balloon containing the fat;*
P_0*: mass of the vacuum flask*

Chemical analyzes: The chemical parameters of the samples taken were determined at the Soil Science Laboratory of the Faculty of Agronomy at Abdou Moumouni University in Niamey.

The chemical analyzes concerned the following parameters: pHwater, total nitrogen, total carbon, total potassium and total phosphorus, calcium; magnesium.

pHwater: The water pH was measured using an electronic pH meter with a glass electrode in the ratio 1/10. To do this

Determination of total nitrogen: The total nitrogen content was determined by the Kjeldahl method (AOAC, 1990). One gram (1g) of the dry bat guano was weighed and introduced into the Kjeldahl flask. A selenium catalyst was added and 20 ml of concentrated H_2SO_4 was also added to the mixture. The digestion flask with the mixture was heated in the DK20 heating digester block starting with a temperature of 80 ° C then 350 ° C. The contents of the digestion vials were heated until the volume was reduced to 3-4 ml. The contents of the digestion flask were cooled and the volume brought to 100 ml in a volumetric flask. Ten (10) milliliters of each sample extract was pipetted into a Kjeldahl still. 20 ml of 40% NaOH was added and distilled. The distillate was collected over 10 ml of 4% boric acid and three drops of indicator mixed in a 250 ml conical flask for 5 minutes. The presence of nitrogen gave a light blue color. Two hundred milliliters (200 mL) of the distillate was titrated with 0.1N HCl until the color changed from light blue to gray and suddenly turned pink. A blank was performed with the sample solution. The weight of nitrogen was calculated as follows: 14 g of nitrogen contained in a milliequivalent weight of ammonia (Black 1965).

The percentage of nitrogen in the sample was calculated as follows:

$$\textbf{Total N\%} = \frac{14x(A-B)xacidconcx100}{1000xl}$$

A = volume of standard HCl used in the titration of the sample
B = volume of standard HCl used in the blank titration
14: atomic weight of nitrogen
l = mass of the sample in grams

Determination of organic carbon: The organic carbon content was determined using the wet dichromate oxidation method. 0.05 g of organic material (guano) was weighed in an Erlenmeyer flask. Ten milliliters of concentrated sulfuric acid, 10 ml of 0.1667 M K2Cr207 and 10 ml of orthophosphoric acid were added. After the addition of 200 ml of distilled water, the solution was left to stand for 30 minutes on an asbestos sheet and titrated again with solutions of FeSO 0.333 M with the diphenylamine indicator Anderson and Ingram (1998).

$$\textbf{\% Organic C=} \frac{(m.eK2Cr2O7 - m.eFeSO4)\ x\ 1.32\ x\ 0.003}{w} \textbf{ x 100}$$

me. = molarity of the solution x ml of solution used
w = weight of the oven-dried sample in grams
0.003 = milli - equivalent weight of carbon in grams (12/4000)
1.32 = correction factor

Determination of potassium: 1g of the ground bat guano was ashed in a muffle oven at 550 ° C for 4 hours. The ash was dissolved in 2M HCl solution, heated and filtered. The filtrate was diluted to 100 ml with distilled water.

Determination of total phosphorus: A 5 ml aliquot of the supernatant digest was pipetted into a 50 ml volumetric flask.

Five (5) milliliters of ammonium molybdate-ammonium vanadate solution was added. The mixture was made up to volume with distilled water to the 50 ml mark and allowed to stand for 30 minutes for color development. A standard curve was developed along with P concentrations between 0.0, 0.8, 1.6, 2.4, 3.2 and 4.0 µg / ml. The absorbance of the blank, control and samples were read on the Jenway 6051 colorimeter at a wavelength of 430 nm. A standard curve was obtained by plotting the absorbance values of the standard solutions against their respective concentrations.

The concentration of phosphorus (P) was determined from the standard curve.

$$\% P = \frac{\text{curvereadx50}}{\text{Wx1000}}$$

w = sample weight
50 = final volume of solution

Determination of total potassium

The potassium in the ash solution was determined using a flame photometer. Standard potassium solutions were prepared with the following concentrations: 0, 2, 4, 6, 8 and 10 µg / ml. The emission values were read on the flame photometer. A standard curve was obtained by plotting the emission values against their respective concentrations.

Total potassium was determined according to the formula below:

$$\% K = \frac{\text{curve read}}{\text{W x100}}$$

with:*w = weight of sample*

Determination of total calcium: Ten milliliters of the ash solution were placed in an Erlenmeyer flask. Potassium hydroxide, buffer solution, 1 ml of triethanolamine and a few drops of potassium cyanide solutions were added. The mixture was titrated with 0.02 M EDTA solution with murexide as an indicator. The determination of total calcium was carried out using the formula below:

$$\% Ca = \frac{V \times 0.02 \times 20}{W}$$

V = ml of EDTA required for titration
0.02 = molarity of EDTA
20 = equivalent weight of Ca (40/2)
W = sample weight

Determination of total magnesium: The concentration of total magnesium in the residue was calculated by subtracting the value obtained from calcium alone from the calcium + magnesium value.

The following formula was used:

$$\% Mg = \frac{V \times 0.02 \times 12}{W}$$

V = ml of EDTA required for titration
0.02 = molarity of EDTA
20 = equivalent weight of Mg (24/2)
W = sample weight

Determination of total calcium and magnesium: A 10 ml aliquot of ash solution was placed in an Erlenmeyer flask. 10 ml of 10% potassium hydroxide, 1 ml of triethanolamine and a few drops of potassium cyanide solutions were added. The mixture was titrated with 0.02 M EDTA solution with callus red as an indicator.

Determination of the rate of organic matter: The determination of the organic matter in the different types of manure was made after removal of water (drying at 105 ° C) and limestone compounds by calcination in a muffle furnace. The difference in weight after calcination represents organic matter. In this case, the percentage of carbon was obtained by dividing the percentage of organic matter by the factor 1.724.

RESULTS AND DISCUSSION

Results

Physical characteristics of Magarawa guano: A closer look at the samples of guano shows that it comes in the form of small sticks of blackish color **figure 2.** Magarawa’ s bats Guano granules are elongated, usually segmented with tiny surface perforations, blunt ends, and made up of shiny fragments (Figure 2.A). The dimensions of the measured fecal granules vary from 3.7 to 4.1 mm in length (Figure 2.B). The diameter varies between 1.1 to 1.8 mm. When dry, to the touch, bat guano is very crumbly and light. When wet, it gives off a strong pungent odor like urine.

Chemical characteristics of Magarawa's guano: The chemical composition of Magarawa's guano is presented in Table I. It appears from this table that Magarawa's guano is weakly basic with total nitrogen contents of almost 10% and total phosphorus of 0.51%. Its organic carbon content is 46%. Its organic matter content is 80% with a C / N ratio of 5. The contents of the microelement Ca, Mg, Al, Fe and Mn vary slightly between 0.05% to 0.3%.

DISCUSSION

Physical characteristics of Magarawa guano: Observation of guano under a magnifying glass has shown that Magarawa's bat guano is in the form of small sticks. These results are similar to those of Gnaspini*et al.,* 2012. These authors have shown that insectivorous bat guano is generally finely granulated and consists of small pieces of insect cuticle sometimes with still adherent material. In other words, this type of guano is usually granulated and consists of the remains of decaying insects. According to the Oregon Department of Agriculture in the US, after studying 16 different samples of guano, the pellet shape of bat guano is related to the content of organic matter. They have shown that guano, which is rich in organic matter, has a granulated form, while guano, which is low in organic matter, has a powdery form. This therefore confirms that Magarawa's guano has a high organic matter content of 81%, hence its granulated form.

pH of bat guano: Study results showed that Magarawa's bat guano has a pH of 7.87; it is basic. This high pH could be explained by the state of the guano analyzed. Indeed, the guano collected during this study was fresh. However, the pH of guano changes according to its age and storage conditions (Audra et al., 2016). So, the fresh guano is slightly basic. Water percolation leaching removes ammonia first.

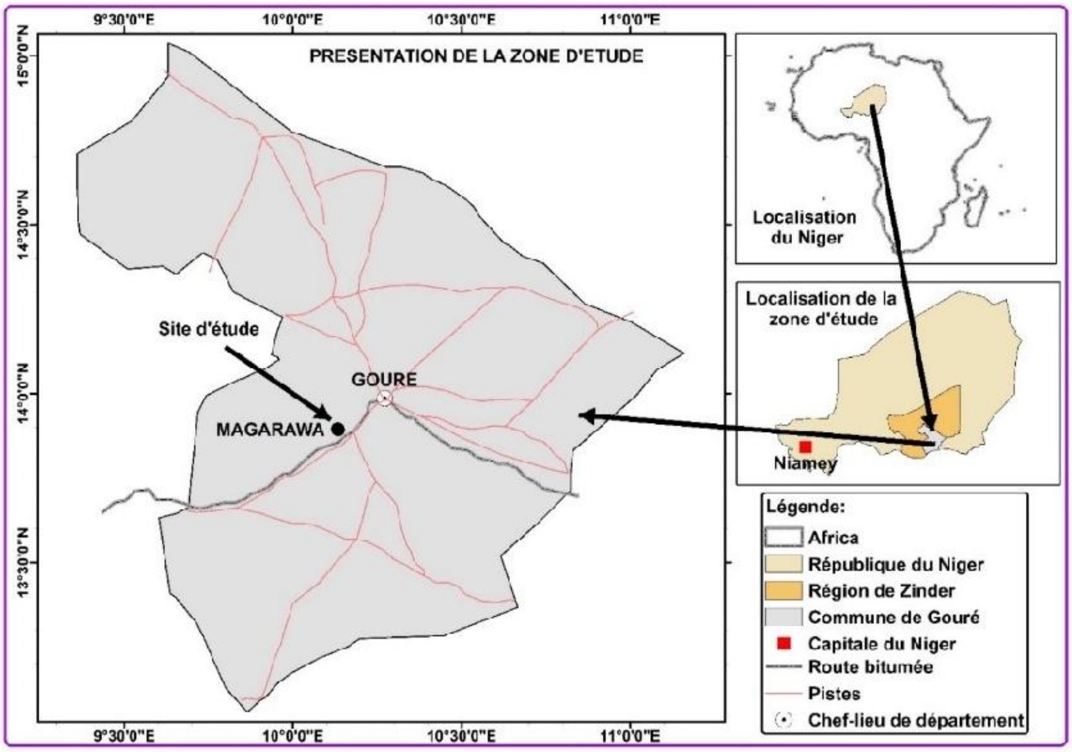

Figure 1. Geographical location of the village of Magarawa within the Department of Gouré.

(A): sample of guano; (B): guano granule.

Figure 2. Physical aspects of Magarawa bat guano:

Table 1. Chemical parameters measured in the Magarawa guano sample

Parameters	Concentration	Parameters	Concentration
pH	7,8±0,8	Dry matter (%)	92,5±2,4
Nitrogen %	9,8±1,1	Minéral matter (%)	6,9±2,3
Phosphorus (mg/kg)	5090±127	Protéin (%)	60,66±8,5
Potassium (mg/kg)	6571±95	Fat (%)	1,14±0,42
total Carbon (%)	46,6±1.34		
Organic matter (%)	80,15±1,15		
C/N	5±0,54		
Calcium (mg/kg)	962±26		
Sodium (mg/kg)	574±48		
Aluminium (mg/kg)	1335±145		
Iron (mg/kg)	1003±241		
Magnesium (mg/kg)	3438±381		
Manganese (mg/kg)	190±43		

In an old guano, percolation of water produces acid solutions that can reach a very low pH between 2 and 4 (Martini, 1993; Mulec *et al*., 2015, Audra *et al*., 2016). According to Mulec *et al*., (2015) the variations in pH for different guano vary between 3.54 and 7.77 depending on their age. The pH decreases with the age of the guano. We remember at this level that the pH of Magarawa's guano is similar to that of many guanos around the world.

Nitrogen content: The results showed that Magarawa's guano has a relatively average nitrogen content of 9.9%. Nitrogen is an essential element in assessing the quality of fertilizers. In fact, guano is part of the category of organic fertilizers also called nitrogen fertilizer because of their high nitrogen content. The nitrogen content of Magarawa guano is between those of desert guano (Young and Holt, 1977) and *Tadarida brasiliensis* (Emerson *et al.,* 2007) which are respectively 8 and 10. However, the nitrogen content of Magarawa's guano is vastly superior to that of guano studied in other studies. Indeed, Magarawa's guano is 7 times richer in nitrogen than the guano studied by Mathur *et al.,* (1990); Young and Holt (1977); Nitron Industries (2000) having a nitrogen content between 1 and 3%. This difference in organic nitrogen of the different guanos can be linked to the way bats eat. Indeed, Studier *et al*. (1994) showed that the guano of insectivorous or omnivorous bats have a higher nitrogen content than that produced by fruit bats.

As guano is a mixture of solid and liquid waste, in particular urine, the high nitrogen content of guano could therefore be linked to the quality of the droppings produced by bats. Indeed, a swarm of about 150 individuals of Myotis daubentoni generates about 36 g of urine every 12 hours (Webb *et al.,* 1994). Evaporation and rapid breakdown by bacteria from the urine generate ammonia (McFarlane et al., 1995; James, 2013). According to some studies, the air in the cave, whose populations can exceed 10 million individuals, can therefore contain between 1000 and 2000 ppm of ammonia (Mizutani *et al.*, 1992; McFarlane *et al.*, 1995). A study carried out on bats from Panama shows nitrogen levels varying between 3 and 8337 mEq / L of urine (Studier and Wilson, 1983)

Phosphorus content: Phosphorus is an essential element for many living organisms. The phosphorus content of Magarawa's guano is 0.5%. This phosphorus level is similar to the 0.7% level found in the guano of the bat *Desmodus rotundus* (blood-eating type species) by Emerson et al. (2007). Mathur *et al.* (1990) ; Young and Holt (1977); Emerson *et al.* (2007); Goveas*et al*. (2006) found phosphorus levels 5 times higher in bat guano *adarida brasiliensis* and *Pteropus giganteus* (frugivorous species) than in Magarawa guano. This difference may be due to the bat's diet. Indeed, Sridhar *et al*. (2006) who concluded that the guano of fruit bats has a higher phosphorus content than that of insectivorous bats. The difference in phosphorus contents could also be explained by the place of storage. The decomposition of guano releases organic acids which will eventually mix with water. These corrosive solutions combined with the high temperature in the cavities will then react with the calcium carbonate of the surrounding area (caves) and form phosphate minerals, mainly hydroxyapatite Ca5 (PO4) 3 (OH) (Poulson and Lavoie, 2000; Audra *et al.*, 2018). Also, the determined phosphorus content of Magarawa's guano is significantly lower than that obtained by Andrew (2016) which is between 7-15%.

It is also lower than that obtained by Sikazwe and Waele (2004) which is 8.41%. This low phosphorus content of Magarawa's guano could be explained by the decomposition of nutrients such as phosphorus which are leached (Zapata, 1995; Bird *et al.,* 2007; Cleary *et al.,* 2017).

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Organic matter content: Organic matter is the backbone of soil fertility. This organic matter is very low in Sahelian soils and to enhance it requires significant external inputs. GM has a high organic matter content (88%) according to the FAO classification. These results corroborate those of Emarson and Rork (2007). The composition of guano in carbon can be due to the diet and also to the geographical position of the production site. Emarson and Rork (2007) reported that bat guano feeding on insects, blood and fruits, containing 88.8, 88.4 and 84.3% organic matter, respectively. In Israel organic matter contents in bat guano of 60% and 53-65% have been found (Shahack-Gross *et al*. 2004). In India, insectivorous bat guano has reported an organic matter content of 45.6% (Sridhar *et al.* 2006). We note that the guano of insectivores is higher in organic matter than the frugivores.

This difference is due to the fact that the carbon contained in the non-digestible components of the fruits consumed by the fruit eaters is often excluded from the dejection. Although fruit bats consume a diet containing relatively high concentrations of structural carbohydrates (for example, cellulose and hemicellulose), they ingest juices and soft fruit pulp differently by filtering the fruits against their palate and spitting out the more fibrous components in the form of ejecta or granules (Kunz and Diaz, 1995; Entwistle and Corp, 1997; Wendeln*et al.,* 2000; Emarson and Rork (2007). While, in guano in insectivores, there is a strong presence of carbohydrates such as chitin (Whitaker *et al.*, 2004). According to the same authors, although chitinase of bacterial origin has been detected in insectivorous bats, these enzymes apparently do not contribute not to the digestion of the heavy parts of chitinous insects by these bats which will end up in the excrements. Apart from variations due to bat species, diets and geographic regions, the organic matter content of the guano deposit also varies with depth. The organic matter content generally decreases with depth in old deposits of guano that has already undergone decomposition (Shahack-Gross *et al.*, 2004; Bird *et al.*, 2007; Wurster *et al*., 2015).

Content of non-essential elements: In addition to macro elements, guano also contains secondary and microelements. As their name suggests, these elements are also necessary for the proper functioning of living organisms. The secondary elements calcium magnesium has respective levels Ca = 0.1% and Mg = 0.3%. The microelements found in this guano are Aluminum (0.1%); Iron (0.1%); and Mn = 0.01%) amount in GM. All these values are lower than those of Sikazwe and Waele, (2004); Sridhar *et al.* (2006); Buliga, (2010); Shetty *et al*, (2013). The variability of the microelement composition of guano can be influenced by external elements. Guano materials especially in old deposits, interact with weathered cave materials to form new authigenic (secondary) minerals largely phosphate with other elements such as aluminum (Al), potassium (K) and iron (Fe) from guano (Gile and Carrero, 1918, Giurgiu *et al.*, 2013, Giurgiu and Tămaş, 2013, Shahack-Gross *et al*., 2004, Tămaş, Mihet, and Giurgiu, 2014).

Lipid and protein composition: The variation in lipid and protein content of guano depends on eating habits, bat species and location (Bailey and Robertson, 1982, Sikazwe and Waele, 2004, Emerson and Roark, 2007). The faecal composition reflects the composition of the biomass ingested (Silva and Chamul, 2000; Kamler and Homolka, 2005, Emerson and Roark, 2007 and Zhao *et al*, 2007). Insects are known to be a rich source of lipids and proteins (Finke, 2002, Marconi *et al,* 2002, Banjo et al, 2006). When an element is ingested in small quantities, physiological mechanisms maximize the assimilation of this element and therefore minimize the fecal concentration of this element and vice versa (Studier *et al,* 1991; Herrera *et al,* 2001). It is also known that the nutritional composition of insects varies with and within the species, stages of development, sex and location (Banjo *et al,* 2006, Xiaomm*get al,* 2010) which is another factor contributing to the variation in protein and fat content. According to the different characteristics one can hypothesize that the bat guano of Magarawa is of the insectivorous type.

Conclusion

Magarawa bat guano in Niger is an organic fertilizer that fertilizes the soil and increases the yields of vegetable crops. However, its physical, chemical and essential composition have not been studied. It is in this perspective that the present study has provided a better understanding of bat guano in the village of Magarawa, both in terms of its physical and chemical composition and its essential elements. It emerged from this study that Magarawa bat guano has a very high organic matter content of 81% and an average nitrogen content of 10%. However, its phosphorus and potassium contents remain low at 0.5% and 0.65% respectively. Despite this, Magarawa's bat guano comes across as a very good organic fertilizer. Although of excellent quality, Magarawa's guano has a low phosphorus content, this defect can be corrected by combining it with another source of phosphorus such as rock phosphate. This composition of magarawa guano gives it the ability to be a rich organic fertilizer comparable to other commercial guanos around the world.

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