Journal of Food and Nutrition Sciences
2022; 10(1): 1-7
http://www.sciencepublishinggroup.com/j/jfns
doi: 10.11648/j.jfns.20221001.11
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

Evaluation of the Mineral Content and Their Bioavailability in *Macrotermes subhyalinus* (Winged, Queen and Soldier)

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To cite this article:
Angaman Djédoux Maxime, Boko Adjoua Christiane Eunice, Kambou Sansan. Evaluation of the Mineral Content and Their Bioavailability in *Macrotermes subhyalinus* (Winged, Queen and Soldier). *Journal of Food and Nutrition Sciences.* Vol. 10, No. 1, 2022, pp. 1-7.
doi: 10.11648/j.jfns.20221001.11

Received: December 2, 2021; **Accepted**: December 20, 2021; **Published**: January 8, 2022

Abstract: Edible insects are an important source of nutrients that can help to cover certain nutritional deficiencies. This is the case with *Macrotermes subhyalinus*, a species of termite widely consumed in Côte d'Ivoire. This study was carried out to assess the mineral content and their bioavailability in the different castes (winged, queen and soldier) of this species. For this, the levels of antinutritional factors, the levels of minerals and their bioavailability were determined. Magnesium and potassium contents vary respectively from 977.18 mg/100g DM to 1405.45 mg/100g DM and from 6658.20 mg/100g DM to 9879.38 mg/100g DM. The winged *M. subhyalinus* had the higher levels of copper (62 mg/100g DM) and manganese (2867 mg/100g DM). While the queen had the highest values for sodium (2158.9 mg/100g DM) and selenium (0.67 mg/100g DM). As for *M. subhyalinus* soldier, it was rich in calcium (3323.4 mg/100g DM), zinc (127 mg/100g DM), iron (2657 mg/100g DM) and molybdenum (0.48 mg/100g DM). Regarding the content of antinutritional factors, the highest values are observed with *M. subhyalinus* soldier in phytate (451.85±28.49 mg/100g DM) and in tannins (35.32±0.98 mg/100g DM). *M. subhyalinus* queen has the highest oxalate content (12.57±0.48 mg/100g DM). The calculated molar ratios vary from 0.02 to 0.9 with oxalate and from 0.09 to 35.57 with phytates. These insects could be recommended for children suffering from micronutrient deficiency malnutrition.

Keywords: Edible Insects, *Macrotermes subhyalinus*, Mineral Content, Bioavailability, Antinutritional Factors

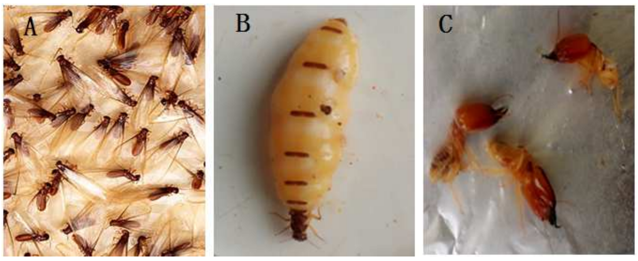
1. Introduction

Food insecurity in Côte d'Ivoire remains a central issue of national concerns. Despite the integration of the fight against malnutrition into national programs designed to promote and support good infant nutrition, the nutritional situation of Ivorian children is deteriorating alarmingly [1]. This phenomenon is perceived in several forms including the chronic and acute form, underweight and mainly micronutrient deficiencies such as iron, zinc, iodine, etc. [2]. This latter form of malnutrition causes weakened immune systems and anemia in children. According to the Demographic Health Survey (2012), three children aged from 6 to 59 months out of four (75%) are anemic: 25% in mild form, 46% in moderate form and 3% in severe form [3]. The causes of these nutritional disorders are linked to the poorly diversified diet, based essentially on tubers, roots and cereals which contribute more than 65% to the daily dietary energy intake [4]. It is moreover to fill these nutrient deficiencies that the country has integrated fortification programs into national policies aimed at strengthening micronutrient fortification strategies, as was the case with the National Multisectoral Nutrition Plan (PNMN) 2019-2020. Besides this, consuming other local foods rich in micronutrients could also help in diet diversification in order to prevent nutritional deficiencies and anemia problems. These include edible insects considered by FAO as viable alternatives to conventional animal proteins [5]. In Côte d'Ivoire 11 species of insects are eaten by more than 60% of the population [6, 7]. Winged adults of *Macrotermes subhyalinus* are the most consumed (29.79%) due to their availability [8]. In the social organization of the species, we also find the queen and the soldiers prized by a section of the population of the country. Unlike the adults which have been the subject of some studies [9], the latter two castes receive less scientific attention. This study aims to evaluate the richness in minerals and their bioavailability in the different castes of *Macrotermes subhyalinus* in order to contribute to their nutritional valuation.

2. Material and Methods

2.1. Material

The sampling equipment consists of a pickaxe for breaking up termite mounds, a digital camera for taking pictures and sterile boxes for collecting the different samples. The biological material is made up of the different social categories of the species *Macrotermes subhyalinus* namely the winged adult, the queen and the soldiers as illustrated in Figure 1.



A: winged; B: queen; C: soldiers

Figure 1. *Different castes of Macrotermes subhyalinus.*

2.2. Methods

2.2.1. Determination of Mineral Content

The mineral content was estimated by dry incineration of a sample of dried powder (5g) in a muffle furnace. Then, the different minerals (copper, zinc, iron, selenium, manganese, molybdenum, calcium, potassium, magnesium and sodium) were quantified by mineralizing 0.5g of the ash obtained in 5 mL of HNO_3/HCl [1:3v:v (nitric acid:hydrochloric acid)] for 2 h under reflux. A 1% dilution was made (0.5g in 50 ml) before analyzing the samples. The concentrations were obtained with an atomic absorption spectrometer (AAS) (Perkinelmer A Analyst 2001, Waltham, Massachusetts, USA). All the assays were carried out from calibration curves.

2.2.2. Determination of Antinutritional Compounds

(i). Determination of Phytate

Phytates were assayed using Wade's reagent [10]. To do this, one (1) g of dried and ground sample was homogenized in 20 mL of HCl (0.65 N) with stirring, for 12 h at room temperature. After centrifugation of the mixture at 12,000 rpm for 40 min, 5 ml of the supernatant was removed and then added to 3 ml of Wade's reagent. Then, this solution was left to stand for 15 min and the Optical Density (OD) was read at 490 nm on a spectrophotometer against a control. A calibration range with sodium phytate at 10 µg/mL was performed.

$$\text{Phytate (mg/100g)} = (OD \times 4)/(0.033 \times me) \quad (1)$$

(ii). Determination of Oxalate Content

The oxalate content was determined according to the method of Day and Underwood [11]. One gram (1g) of sample was weighed and 75ml H_2SO_4 (0.1N) was added. The contents of the mixture were mixed thoroughly for an hour of extraction and stirred constantly using a mechanical stirrer. Then the solution is filtered and 25 ml of the filtrate is titrated with 0.1N hot $KMnO_4$ (80–90°C) until a purple color is observed at the end point. The titer value was then multiplied by 0.9004 to give the result expressed in mg/g. The calculation of the oxalate content was as shown in the equation below:

$$\text{Oxalate (mg/100g)} = \text{Title value} \times 0.9004 \quad (2)$$

(iii). Determination of Tannins

The total tannins of the samples were determined according to the Folin-Denis method [12]. To do this, the Folin-Denis reagent was boiled for 2 hours. After cooling, the solution was transferred to a 1L volumetric flask and the volume was adjusted. This solution is then filtered through a 0.45 µm syringe and stored in a brown bottle. At this step, the standard curve modeling solutions were prepared from a series of 7 dilutions of tannic acid in water using a stock of 0.05 mg/ml solution. Also 0.2 g of sample is suspended in 5 ml of solution B in a plastic tube. The tubes are shaken for 40 minutes and the suspension is filtered. One ml of the filtrate is added to 2.5 ml of Folin-Denis reagent, 5 ml of solution A then the total volume is made up to 50 ml with Milli-Q water. The solutions were incubated for 30 minutes at room temperature and the absorbance is read at 700 nm using a spectrophotometer.

2.2.3. Calculation of Phytate/Mineral and Oxalate/Mineral Molar Ratios

The phytate/mineral and oxalate/mineral molar ratios are considered to be good predictors of the bioavailability of minerals in food [13]. This availability is illustrated through the calculation of these different ratios.

2.2.4. Statistical Analysis

All measurements were performed in triplicate and data averages were statistically analyzed using R software (R × 64 4.1.2). One-way analysis of variance (ANOVA) was performed to compare the means. The differences were considered significant for p values ≤ 0.05.

3. Results and Discussion

3.1. Mineral Content

Figure 2 below shows the PCA Biplot analysis of the mineral content of the different samples. The first two dimensions of the figure express 100% of the total inertias of the set of data; this means that the variability of individuals (or variables) is very well represented by the plan. In the foreground of the figure with respect to the y-axis, we observe a correlation of winged *M. subhyalinus* with Manganese (Mn) and Copper (Cu), which means that this category is distinguished from others by its Manganese (2867 mg/100g DM) and Copper (62 mg / 100g DM). The contents obtained are higher than those [14] which are 3250.1 ± 0.07 mg/kg for manganese and 47 mg/kg for copper on flour of the same caste (*M. subhyalinus* winged). As for *M. subhyalinus* queen, it is more correlated with Sodium (Na) and Selenium (Se) with respective contents of 2158.9 mg/100g DM and 0.67 mg/100g DM. Compared to the nutritional reference values [15] whose needs are recommended at 1500 mg/kg/day for sodium and 0.3 mg/kg/day for selenium, *M. subhyalinus* queen could provide a sufficient nutritional amount of these minerals. The analysis of Figure 2 shows that *M. subhyalinus* soldier is very rich in Calcium (3323.4 mg/100g DM), Iron (2657 mg/100g DM), Zinc (127 mg/100g DM) and Molybdenum (0.48 mg/100g DM). These values are higher than those obtained by the previous study [16] on raw *Macrotermes bellicosus* which are 152.11±0.98 mg/100g DM for calcium, 7.9±0.62 mg/100g DM for iron and 19.70±0.32 mg/100g DM for zinc. This result highlights the importance of *M. subhyalinus* soldier in the food sector in view of the essential role that these different minerals play in the body. Indeed, calcium is the most abundant mineral in the body, 99% of which is located in the bones and teeth [17]. It provides bone accretion, nerve conduction, muscle contraction and hemostasis [18]. Iron has a major role in the functioning of cells. It is a major constituent of hemoglobin, a protein in red blood cells responsible for transporting oxygen from the lungs to the body's cells. Its deficiency is common worldwide, especially in pregnant women and people with anemia [19]. Consuming *M. subhyalinus* soldier could easily help to compensate for these iron deficiencies in order to provide a solution to the problems of anemia. Zinc for its part, has an essential role for the functionality of more than 300 enzymes, for the stabilization of DNA and for the expression of genes. Zinc deficiency leads to slower growth and development in newborns and children [20]. For this high zinc content (127 mg/100g DM) this category of *M. subhyalinus* could be recommended for children who have shown growth retardation.

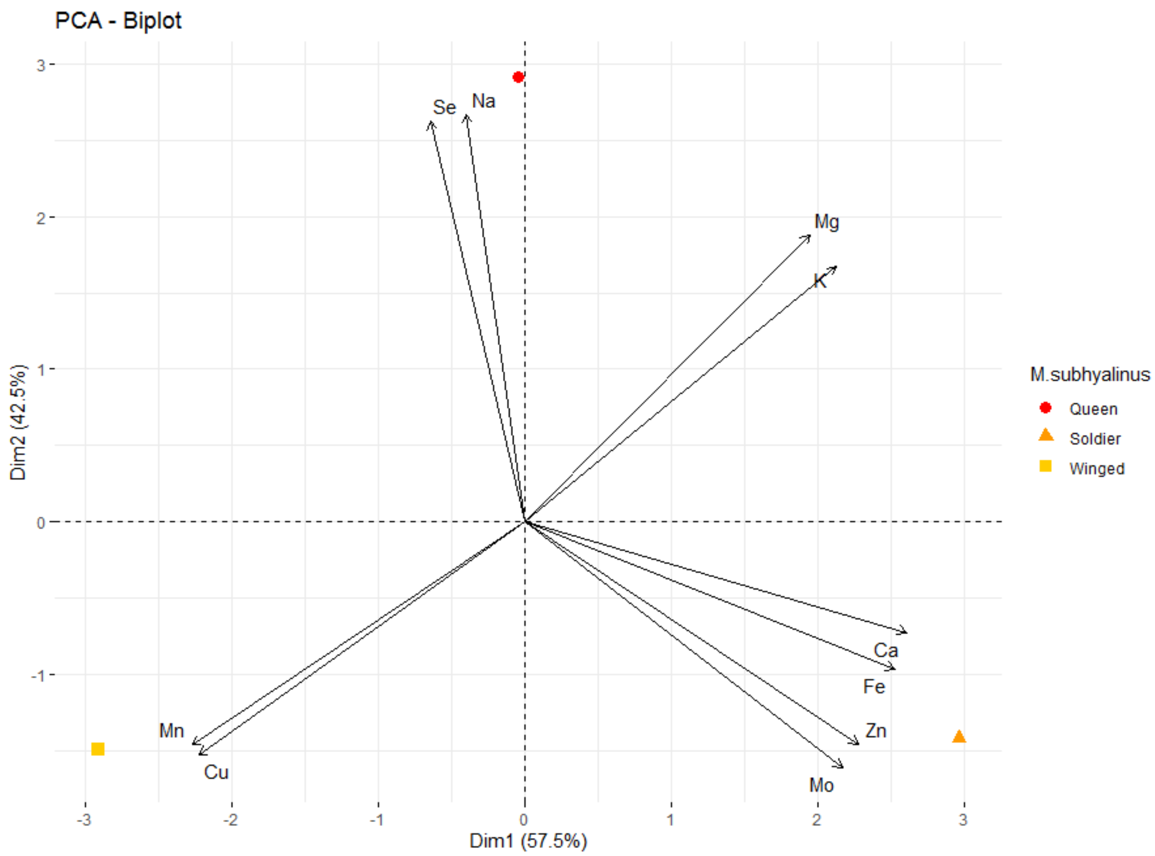


Figure 2. *PCA-Biplot analysis of the mineral content of the different samples.*

3.2. Antinutritional Factor Levels and Mineral Availability

The levels of antinutritional compounds of the different castes of *M. subhyalinus* are shown in Figures 3, 4 and 5. The phytate/mineral and oxalate/mineral molar ratios are considered to be good predictors of the bioavailability of minerals in food [13]. This availability is illustrated by Table 1 below through the calculation of these different ratios. The oxalate content of the different samples are relatively low and vary from 6.21±1.09 mg/100g DM for *M. subhyalinus* winged to 12.57±0.83 mg/100g DM for *M. subhyalinus* queen. The one-way Anova test ($p \leq 0.05$) shows identical levels for *M.*

subhyalinus queen (12.57±0.83 mg/100g DM) and *M. subhyalinus* soldier (11.46±0.41 mg/100g DM) (Figure 3). These values are lower than those obtained by [21] on the most consumed leafy vegetables in the city of Daloa (Center-West, Côte d'Ivoire) which vary from 44.20±1.27 mg/100g DM to 64.75±0.52 mg/100g DM. In addition, all calculated [oxalate]/[minerals] molar ratios were below the maximum critical values (Table 1). This result shows that the oxalate contents of the samples analyzed cannot influence the bioavailability of the minerals contained in these foods [22]. In terms of phytate content, *M. subhyalinus* queen (92.19±6.19 mg/100g DM) and M. subhyalinus winged (94.14±3.16 mg/100g DM) have statistically identical levels ($p \geq 0.05$). A high value is observed with *M. subhyalinus* soldier (451.8±28.49 mg/100g DM) (Figure 4). These contents remain lower than those obtained by the previous study [23] in cereals which are 762 mg/100 DM for corn, 1084 mg/100g DM for rice and 925 mg/100g DM for sorghum. The [Phytate]/[Fe] and [Phytate]/[Mg] molar ratios of the three samples vary respectively from 1.70 to 7.97 and from 0.65 to 3.46 (Table 1). These ratios are greater than the maximum critical values defined by [24] which are 0.40 for [Phytate]/[Fe] and 0.24 for [Phytate]/[Mg]. This indicates a low bioavailability of the two minerals (Iron and Magnesium) despite their high contents in the samples. With the exception of *M. subhyalinus* soldier, the other samples have [Phytate]/[Zn], [Phytate]/[Ca] and [Phytate]/[K] ratios lower than the maximum critical values defined by [23, 24]. Therefore these minerals (Zinc, Calcium and Potassium) from *M. subhyalinus* Winged and *M. subhyalinus* Reine have a high bioavailability and would be easily absorbed by the body. On the other hand, the minerals of *M. subhyalinus* soldier would be difficult for the body to absorb because of the high phytate content of this caste of *M. subhyalinus*. The tannin contents differ from sample to sample (Figure 5). M. subhyalinus soldier shows the highest content (35.32±1.49 mg/100g DM) followed by *M. subhyalinus* queen (26.98±0.27 mg/100g DM) and winged *M. subhyalinus* (08.01±0.44 mg/100g DM). These contents are relatively high and could constitute an unfavorable factor absorption of certain nutrients such as protein and iron. Indeed, tannins can form complexes with proteins to reduce their digestibility and negatively impact the absorption of minerals such as iron, copper and zinc as well as their reserves [25, 26]. However, these insect tannins should attract much more attention as several researchers have claimed that the polyphenols are mainly of plant origin [27, 28]. They can be tested in the medical field as is the case with vegetable tannins in the fight against wounds, hemorrhoids, diarrhea and gastroenteritis [29].

Table 1. *Phytate/mineral and oxalate/mineral molar ratios of Macrotermes subhyalinus.*

	Winged	Queen	Soldiers	*Critical value
[Phytate]/[Fe]	7.97	1.98	1.70	0.15
[Phytate]/[Zn]	10.82	10.97	35.57	15.00
[Phytate]/[Ca]	0.43	0.37	1.35	0.50
[Phytate]/[Mg]	0.96	0.65	3.46	0.24
[Phytate]/[K]	0.14	0.09	0.47	0.25
[Oxalate]/[Fe]	0.52	0.24	0.04	2.5
[Oxalate]/[Zn]	0.71	1.49	0.90	2.5
[Oxalate]/[Ca]	0.02	0.05	0.03	2.5
[Oxalate]/[Mg]	0.06	0.08	0.08	2.5
[Oxalate]/[K]	0.02	0.05	0.03	2.5

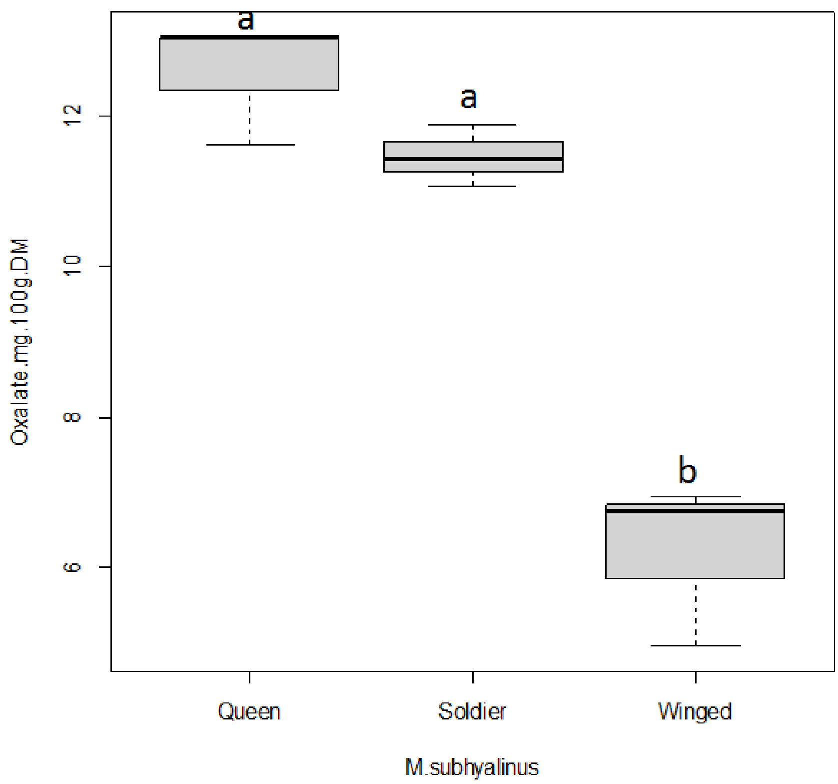


Figure 3. *Oxalate content of different castes of M. subhyalinus.*

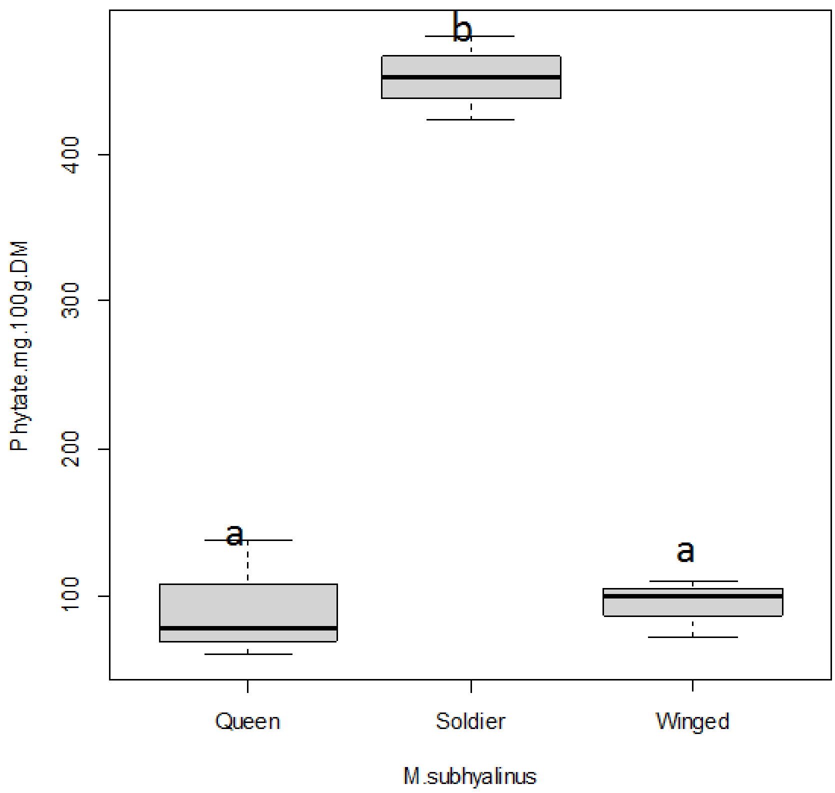


Figure 4. *Phytate content of different castes of M. subhyalinus.*

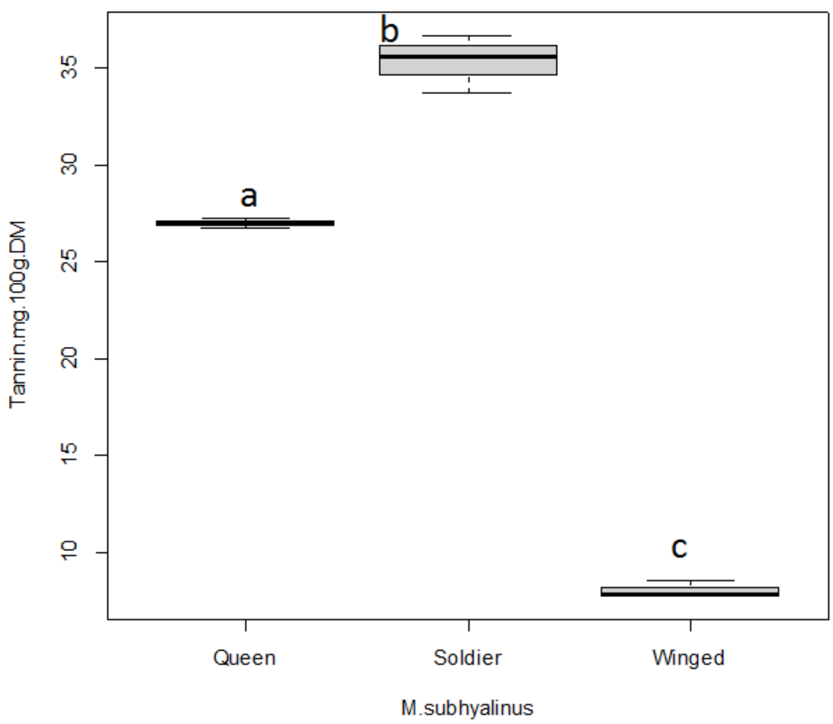


Figure 5. *Tannins content of different castes of M. subhyalinus.*

4. Conclusion

The present study on the edible termite species *Macrotermes subhyalinus* was carried out with the objective of evaluating the mineral richness and their bioavailability in the different castes (winged, queen and soldier) of this species. For this, the levels of antinutritional factors, the levels of minerals and their bioavailability were determined. It emerges from this study that the different categories of *Macrotermes subhyalinus* (winged, queen and soldier) have a high mineral content which varies according to the castes. The winged *M.*

subhyalinus caste is distinguished by its richness in copper and manganese with respective contents of 62 mg/100g DM and 2867 mg/100g DM. The Queen's caste has the highest values for sodium (2158.9 mg/100g DM) and selenium (0.67 mg/100g DM). As for *M. subhyalinus* soldier, it is rich in calcium (3323.4 mg/100g DM), zinc (127 mg/100g DM), iron (2657 mg/100g DM) and molybdenum (0.48 mg/100g DM). The magnesium and potassium contents of the three castes vary respectively from 977.18 mg/100g DM to 1405.45 mg/100g DM and from 6658.20 mg/100g DM to 9879.38 mg/100g DM. Regarding the content of anti-nutritional factors, the highest values are observed with *M. subhyalinus* soldier in phytate (451.85±28.49 mg/100g DM) and *M. subhyalinus* queen in oxalate (12.57±0.48 mg/100g DM). The calculated molar ratios are relatively low, which implies availability of minerals at the level of the samples studied. These insects could be recommended for children suffering from micronutrient deficiency malnutrition. Formulations of cereal flour enriched with these insects could be developed and characterized.

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