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# Selected Chemical Properties, Microbial Activity and Biomass of Soils Amended with Aqueous Neem Leaf Extract

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**Abstract**

With declining fertility levels of soils and the high cost of agricultural inputs, such as commercial fertilizers and pesticides, the use of organic inputs has increased in Zambia. While neem products have been shown to improve soil fertility status, several negative effects on soil organisms have also been cited. The negative effects have been attributed to several secondary metabolites produced by the neem plant. In Zambia, neem leaf extract is applied by small scale farmers to enhance soil fertility and promote crop productivity. This study reports the suitability of aqueous neem leaf extract as a soil amendment and its effect on soil microbial biomass and activity in local soils. Neem leaves were characterized before being used to prepare aqueous neem extract in the concentrations 2, 5, 10, 15, and 20 % in water. The extract was characterized for selected mineral components and then applied to 5 kg of soil on a weekly basis for five weeks. Each week, for ten weeks, the effect of the extract on microbial biomass and activity were determined using the Chloroform Fumigation and Incubation (CFI) and soil respiration methods, respectively. Selected soil chemical characteristics were determined at the start and end of the experiment. Results indicated that the chemical composition of the neem leaves was comparable to that observed by others and was similar to that of other tree leaves used for preparing leaf extracts. Amending soils with neem did not significantly improve selected chemical properties but only marginally increased soil calcium levels. Neem leaf extract enhanced soil microbial activity up to 10 %, but showed inhibitory effects at 15 and 20 % concentrations. Microbial biomass was also depressed by neem leaf extract at 20 %. The reduction in both microbial activity and biomass was possibly due to the negative effects of the neem secondary metabolites in the leaf extract at these higher concentrations. Although the application of neem leaf extract at 10 % percent or higher can inhibit both microbial biomass and activity, some mineralizable components in the extracts can support growth and activity of some microorganisms in the soil. Based on these results, the application of neem leaf extract at 10 % percent or higher can inhibit both microbial biomass and activity and marginally improve soil Ca levels. The use of neem leaf extract can therefore be of benefit to soils with critically low levels of Ca.

**Keywords:** Microbial biomass, microbial activity, neem, aqueous extract, soil chemical properties

## 1. Introduction

Neem *(Azadirachta indica*) is a tropical evergreen tree which belongs to the Mahogany family (Ogbuewe et al., 2011). This tree has different medicinal and agricultural uses. Neem products such as leaf powder, seed powder, extracts, saw-dust, oil and oil cake are used as bio-medicines, pesticides, soil amendments and livestock feed (Akhtar, 1998, 1999; Hassan, Galti, & Ali., 2015; Ogbuewe et al., 2011; Sarawaneeyaruk, Krajangsang, & Pringsulaka, 2015).

In agriculture, neem plant products have been used to promote seed germination, to improve soil quality and to protect various crop species from different classes of insects (Sayed, Salem, & Ali, 2011). As organic fertilizers, neem leaves, neem seed cake, and leaf, seed and bark extracts have been shown to improve some chemical characteristics of soils. Particularly, amending soils with neem products has resulted in an improvement in soil pH, total nitrogen, ammonium nitrogen, exchangeable bases, micronutrients and electrical conductivity (Aduloju, Adelana, & Shuaib, 2013; Elnasikh, Osman, & Sherif, 2011). These changes in soil fertility have been attributed to the chemical composition of the different parts of the neem plant used as soil amendments (Solomon, Okon, & Umoetok, 2008; Elnasikh et al., 2011); which are sometimes richer in some mineral components than farmyard manure or sewage sludge. As pesticides, neem products have been shown to be able to control ants (termites) and nematodes (Akhtar, 1994) and to protect plants from insect pests.

Neem products possess anti-cholinesterase, anti-septic, anti-pyretic, anti-inflammatory, anti-bacterial and anti-fungal properties due to more than 100 active secondary compounds called triterpenoids or limonoids that they possess (Djibril et al., 2015; Koul & Wahab, 2004). Based on the properties of these compounds, studies have suggested varied effects on both beneficial and non-beneficial rhizospheric soil organisms. For example, the application of neem cake has been shown to offset the natural balance of some microorganisms by increasing or decreasing their populations (Akhtar, 1994). Some studies *in vitro* have shown direct effects on particular organisms such as nitrifying bacteria, fungi and carbon decomposers (Elnasikh et al., 2011; Sayed et al, 2011; Solomon et al., 2008). While studies on the effects of using neem products as soil amendments on specific organisms has enhanced knowledge in the field of soil microbiology, the use of soil microbial biomass as an indicator of change would provide a more sensitive and much quicker way of assessing these effects.

Soil Microbial biomass refers to the living part of soil organic matter but does not include plant roots and soil animals bigger than 5 x $10^3$ $\mu m^3$ in size (Abbas, Akmal, Khan, & Hassan, 2014; Azam, Farooq, & Lodhi, 2003) Microbial biomass comprises 2-6 % of total soil organic matter and 1-3 % of soil organic carbon (Baaru, Munngendi, Bationo, Verchot, & Waceke, 2007; Martens, 1995).

Soil microbial biomass is affected by many soil management factors. Studies have shown that the application of compost, straw and natural amendments results in an increase in soil microbial biomass (Araujo & Melo, 2010; Malik, Khan, Marschner & Hassan, 2013). On the other hand, fertilizers and pesticides affect microbial biomass in various ways depending on the length of time under consideration, the rate of application and the predominant soil organisms (Subhani, El-ghamry, Chanyong, & Jianming, 2000). Soil microbial biomass is particularly sensitive to changes in soil management systems, soil physical and chemical properties, season, cropping pattern and tillage, among other factors (Abbas et al., 2014; Kara & Bolat, 2008; Jenkinson, 1988; Lupwayi et al., 2004) and provides an alternative for assessing soil health.

With the declining fertility levels of soils in Zambia and the high costs of agricultural inputs, such as fertilizers and pesticides, the use of organic inputs is being promoted by both government and non-government entities. As a result, organic compounds such as neem leaf extract are now being used by small scale farmers in Zambia to improve soil fertility and promote crop productivity. The effect of using neem leaf extract on Zambian soils has not yet been elucidated. We report here the suitability of aqueous neem leaf extract as a soil amendment and its effect on soil microbial biomass and activity.

## 2. Materials and Methods

### *2.1 Site Description and Soil Sampling*

Soils used in the study were collected from Chililabombwe District of agro-ecological region III of Zambia at S12.3667$^o$ and E027.8278$^o$. This region receives greater than 1000 mm of rainfall annually with an average temperature ranging from 16 to 23 $^oC$ and a cropping season of six (6) months lasting from November to March.

Soil sampling was done following the Grid method (Wollenhaupt &Wolkowski, 1994). A total of 60 sub-samples were randomly collected to a depth of 20 cm and thoroughly mixed to make a homogenized composite sample. The composite sample was then divided into two sub-samples; one sub-sample was subjected to chemical and physical characterization and another sample was carried on ice in cooler boxes and kept refrigerated until potted for the greenhouse experiment.

### *2.2 Soil Characterization*

The soil was air dried and passed through a 2 mm sieve before it was analysed for the following: soil reaction (pH), total nitrogen, available phosphorus, soil organic matter, electrical conductivity, exchangeable bases and cation exchange capacity, bulk density and texture. Soil reaction (pH) was measured in 0.01 M $CaCl_2$ using a soil:solution ratio of 1:2.5 using glass-calomel electrodes connected to a pH meter (Van Reeuwijk, 1992). Soil texture, available phosphorus (P) and soil organic carbon (SOC) were determined using the Hydrometer (Day, 1965), Bray 1 (Olsen & Sommers, 1982) and Walkley and Black chromate reduction (VanRanst, Verloo, Demeyer, & Pauwel, 1999) methods, respectively. Calcium (Ca), magnesium (Mg), potassium (K), and cation exchange capacity (CEC) were determined using the ammonium acetate method. Electrical conductivity (EC) was determined using soil-water extraction method (Doll & Lucas, 1975). Soil bulk density was determined using the Paraffin Clod method of Blake (1965).

*2.3 Sampling and Chemical Characterization of Neem Leaves*

Neem leaves were collected from Kasisi Agricultural Training Centre in Chongwe District in agro-ecological region II of Zambia, located 30 km East of Lusaka. The geographical location of Kasisi Agricultural Training Centre is approximately 15$^o$10'25.30" South and 28$^o$ 31'55.12" East and 1, 214 m above sea level.

Young fresh neem leaves were randomly collected from four sample trees, approximately ten years old. The leaves were mixed thoroughly to make a homogenized composite sample before putting in paper bags. Four replicate samples of neem leaves were then oven dried at 60 $^o$C for 72 hours and analyzed for total nitrogen (modified Kjeldah method; Kalra& Maynard, 1991) and for phosphorus, magnesium, potassium and calcium using the Dry Ashing method (Doll & Lucas, 1975).

*2.4. Preparation and Chemical Characterization of Aqueous Neem Leaf Extract*

The young fresh neem leaves collected from the four sample trees were mixed thoroughly to make a homogenous composite sample and apportioned into 0.4, 1, 2, 3 and 4 kg sub- samples. To arrive at different concentrations, each sub-sample was placed in a securely tied sack and immersed in a drum containing 20 liters of water. The concentrations of the extract based on the weight of leaves and volume of the water used to make the tea were 2, 5, 10, 15 and 20 %, respectively. For example, 1 kg of sub-sample in 20 liters of water was equivalent to the 5 % concentration.

The drums were covered with polythene bags to prevent any foreign material from falling in and the mixture was vigorously stirred on a daily basis to allow proper leaching of the nutrients from the leaves into water. The sacks with the leaves were carefully removed after 14 days; to remove any debris, the extract was passed through a 2 mm sieve.

In order to characterize the aqueous neem leaf extracts, laboratory analyses methods for water and liquid organic manure (Chapman and Pratt, 1961) were used to analyze for N, P, K, Ca, Mg, pH and EC.

*2.5 Determination of Microbial Activity and Biomass in Aqueous Neem Leaf Extract Amended Soil*

To determine the effects of the different concentrations of aqueous neem leaf extract on soil microbial biomass and activity, the extract was applied to 5 kg of soil in a pot at a rate of 3 600 l/ha every week for 5 consecutive weeks. This was done following the practice of small-scale farmers in Zambia.

The five concentrations of aqueous neem leaf extract and a non-amended control (tap water) were applied to soils in pots arranged as a greenhouse experiment in a Completely Randomized Design (CRD) with four replications. Every week for 10 weeks, 100 g of soil from each pot (top 2 cm) was removed and analyzed for soil microbial biomass and activity using the Soil Respiration and Chloroform Fumigation and Incubation methods, respectively.

*2.6 Determination of Changes in Selected Soil Chemical Properties due to Neem Leaf Extract Amendment*

After the 10 weeks, the soil remaining in the pots was collected and analyzed for some selected chemical properties according to the methods described in Section 2.2.

*2.7 Data Management and Analyses*

To determine the effects of aqueous neem leaf extract on soil chemical and biological characteristics, all data were subjected to Analysis of Variance (ANOVA) at 95 % level of confidence using the SAS Package version 9.1. The means were separated and compared using Least Square Difference test at the same confidence level.

## 3. Results

*3.1 Nutrient Composition of Neem Leaves and Aqueous Neem Leaf Extract*

Table 1. Nutrient composition of neem leaves

| Nutrients | Leaf content (%) |
|---|---|
| Nitrogen | 1.50±0.05 |
| Phosphorus | 0.09±0.01 |
| Potassium | 1.77±0.10 |
| Calcium | 2.58±0.29 |
| Magnesium | 0.35±0.14 |

Mean values of four replicates ± Standard Error of means.

Of the nutrients analyzed in the leaves of neem, the highest was calcium (2.58 %) followed by potassium (1.77 %), nitrogen (1.5 %) magnesium (0.4 %) and lastly phosphorus (0.1 %) (Table 1).

The contents of various chemicals in the aqueous neem leaf extract increased with concentrations from 0 – 20 % (Table 2). The concentration of ammonium-nitrogen, nitrate-nitrogen, phosphorus, potassium, calcium and magnesium was 84, 63, 33, 34, 63.1, and 96 % higher in 20 % aqueous neem leaf extract than in the control (water). The levels of ammonium-nitrogen were in all cases more than 3 times higher than the nitrate-nitrogen. The EC also increased by a large magnitude (83 %); while pH increased by 1 unit which is equivalent to 100 fold from 0 to 20 %. There were statistical differences among the various concentrations for each of the chemical characteristics measured.

Table 2. Chemical composition of neem leaf extract

| Concentration (%) | $NH_4$-N | $NO_3$-N | P mg/L | K | Ca | Mg | pH Cacl$_2$ | EC mS/cm |
|---|---|---|---|---|---|---|---|---|
| 0 | 15.8d* | 4.6d | 0.2c | 3.5e | 1.4d | 0.3b | 7.1b | 0.7e |
| 2 | 21.7d | 6.0cd | 0.2bc | 35.4d | 1.5cd | 1.5b | 7.9a | 0.8e |
| 5 | 25.2dc | 6.4cd | 0.3ab | 54.9c | 1.5cd | 6.1a | 7.9a | 1.4d |
| 10 | 34.7c | 7.7bc | 0.3a | 59.9c | 1.8bc | 6.3a | 7.9a | 2.3c |
| 15 | 77.7b | 8.8b | 0.3a | 67.9b | 2.0b | 7.0a | 7.9a | 3.0b |
| 20 | 103.6a | 12.6a | 0.3a | 116.0a | 3.8a | 7.4a | 8.1a | 4.0a |
| LSD($p \leq 0.05$) | 10.7 | 2.0 | 0.1 | 5.3 | 0.40 | 2.4 | 0.3 | 0.1 |
| CV (%) | 15.6 | 17.9 | 20.0 | 16.3 | 13.6 | 30.2 | 12.8 | 22.4 |

*Means followed by the same letter within a column are not significantly different

### *3.4 Changes in Selected Soil Chemical Characteristics due to Amendment with Aqueous Neem Leaf Extract*

Generally, amending soils with aqueous neem leaf extract with up to 20 % concentration did not have significant effects on selected soil chemical properties. Specifically, only calcium, was significantly affected (Table 3). The concentration of calcium increased by approximately 5% from 8.1 to 8.5 cmol+/kg across all neem extract concentrations. Magnesium, soil organic carbon, pH, available phosphorus, EC and CEC were not significantly affected; concentrations after amendment with leaf extract were not different from the initial concentrations (Table 3).

Table 3. Changes in P, K, Mg, Ca, OM, CEC, EC and pH of soil due to aqueous neem leaf extract at different concentrations (n = 4)

| Aqueous extract concentration (%) | Initial | 0 | 2 | 5 | 10 | 15 | 20 | LSD ($p \leq 0.05$) | CV (%) |
|---|---|---|---|---|---|---|---|---|---|
| K (cmol+/kg) | 0.3ab* | 0.2b | 0.3ab | 0.3ab | 0.2b | 0.2b | 0.2b | 0.1 | 19.2 |
| Mg (cmol+/kg) | 0.1b | 0.2ab | 0.2ab | 0.2ab | 0.1b | 0.2ab | 0.1b | 0.04 | 16.9 |
| Ca (cmol+/kg) | 8.1b | 8.1b | 8.5a | 8.5a | 8.5a | 8.5a | 8.5a | 0.1 | 10.7 |
| OM (%) (ns) | 1.6a | 1.5a | 1.3a | 1.3a | 1.3a | 1.6a | 1.3a | 1.0 | 15.5 |
| CEC (cmol+/kg) (ns) | 8.0a | 9.3a | 7.8a | 8.8a | 8.8a | 8.5a | 8.5a | 2.7 | 21.1 |
| EC (mS/cm@25$^0$C) (ns) | 0.1a | 0.2a | 0.2a | 0.1a | 0.2a | 0.1a | 0.1a | 0.1 | 22.3 |
| pH ($CaCl_2$) (ns) | 6.4a | 6.6a | 6.4a | 6.5a | 6.5a | 6.6a | 6.5a | 0.2 | 12.0 |
| P (mg/kg) (ns) | 0.1a | 0.5a | 0.2a | 0.6a | 0.5a | 0.5a | 0.6a | 0.5 | 23.5 |

*Means in a row followed by the same letter are not significantly; ns-non significant at 95% CL.

### *3.2 Effect of Aqueous Neem Leaf Extract on Soil Microbial Activity*

Cumulative soil microbial activity was measured at 5 and 10 weeks of amending soils with neem leaf extract. Soil microbial activity ranged from 29 to 39 $CO_2$-C mg/kg wet soil /day at week 5 and from 58 to 72 $CO_2$-C $CO_2$-C mg/kg wet soil /day at week 10. At week 10, soil microbial activity was highest when neem leaf extract was amended at 10 % concentration and was lowest when the concentration was increased to 20 % (Figure 1). A similar pattern was observed at week 5. In general, microbial activity tended to increase with concentration from 0 up to 10 % and then declined with concentration thereafter at both weeks 5 and 10. Figure 2 depicts the magnitude of change in soil microbial activity from the control. The highest positive change is shown at 10 %, while the negative change is shown at 15 and 20 %.

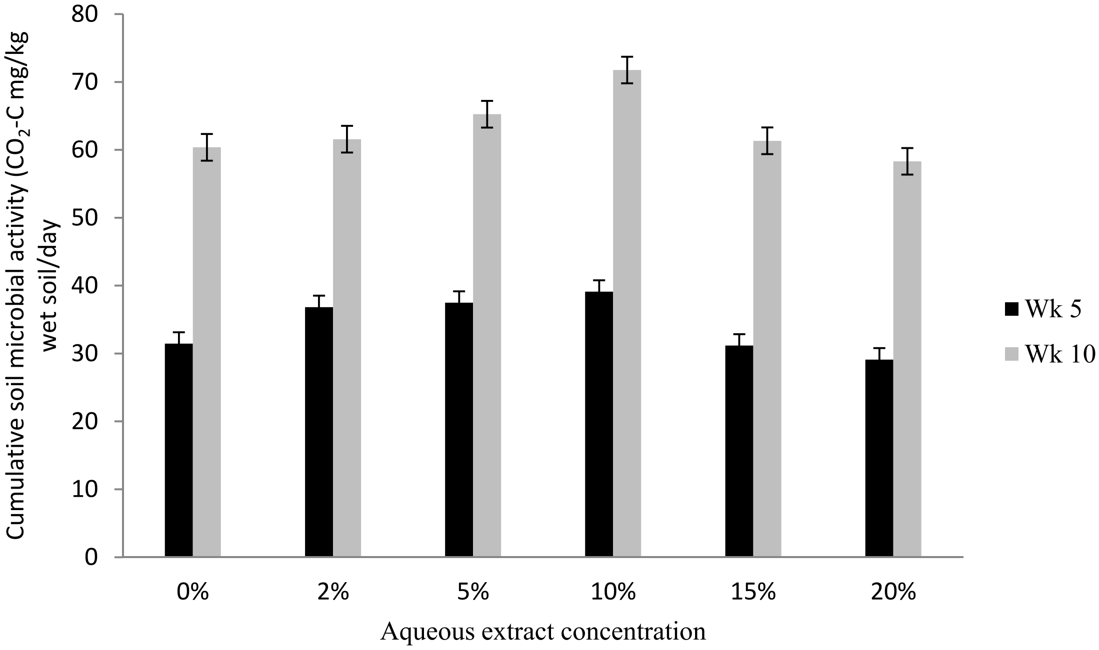


Figure 1. Cumulative soil microbial activity at 5 and 10 weeks of incubation of soils amended with 2, 5, 10, 15 and 20 % aqueous neem leaf extract

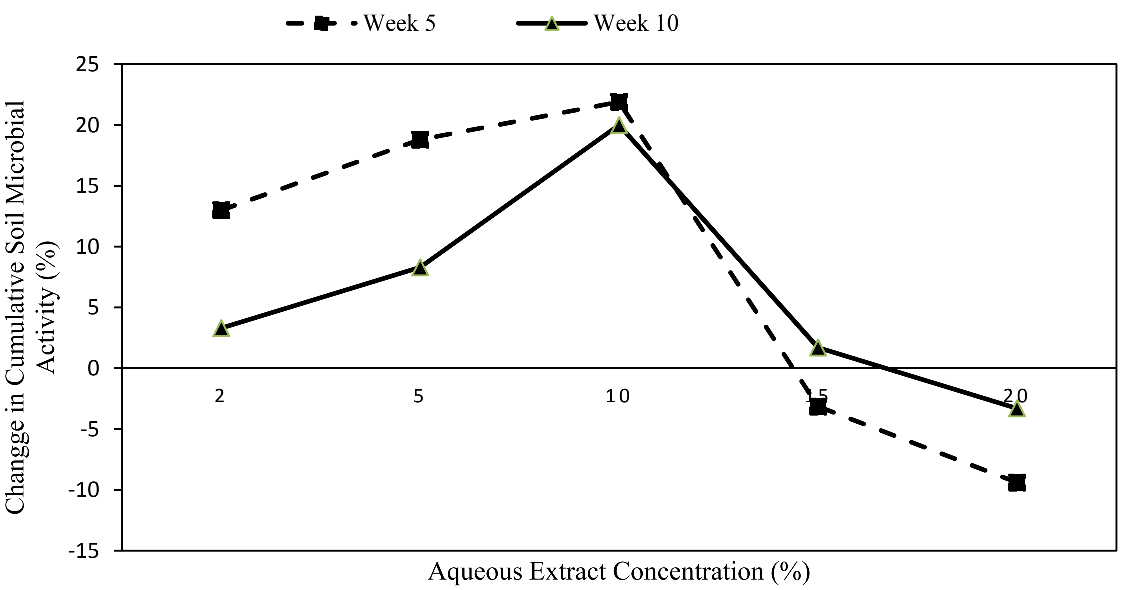


Figure 2. Change in cumulative soil microbial activity from 0 to 20 % aqueous neem leaf extract at 5 and 10 weeks of neem leaf extract amendment

### *3.3 Effect of Aqueous Neem Leaf Extract on Soil Microbial Biomass*

Cumulative soil microbial biomass depended on the concentration of the aqueous neem leaf extract. At week 5, soil microbial biomass ranged from 27 to 36 $CO_2$-C mg/kg wet soil, while at week 5, the range was from 55 to 74 $CO_2$-C mg/kg wet soil. The general trend was that microbial biomass was lowest at 2 % and rose to its highest at 15 % without any significant reduction at 20 % at both weeks 5 and 10 (Figure 3). Figure 4 shows that microbial biomass initially reduced and then increased and reduced again gently after 15 %.

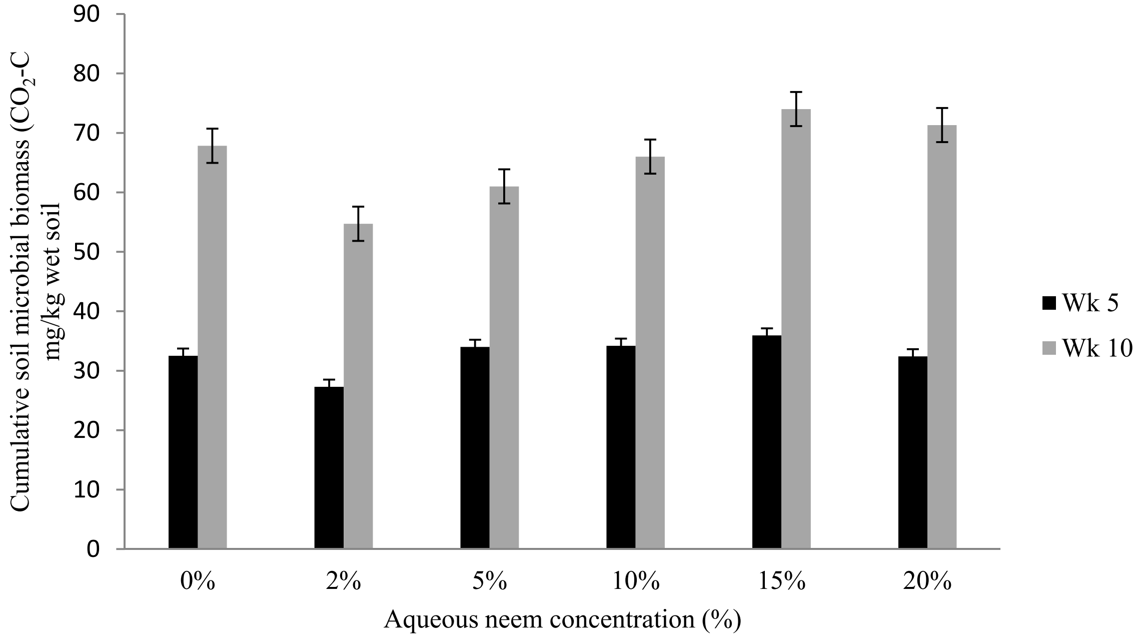


Figure 3. Cumulative soil microbial biomass at 5 and 10 weeks of incubation of soils amended with 2, 5, 10, 15 and 20 % aqueous neem leaf extract

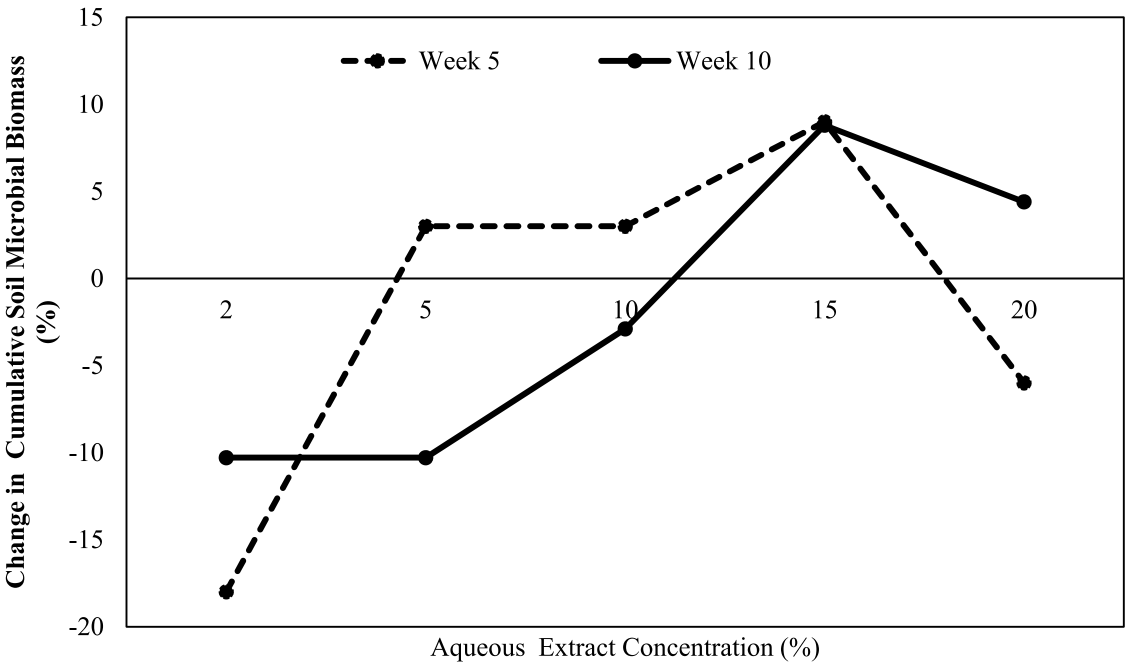


Figure 4. Change in cumulative microbial biomass from 0 to 20 % aqueous neem leaf extract at 5 and 10 weeks after amendment

## 4. Discussion

The composition of neem leaves used for the preparation of neem leaf extract was comparable to that reported earlier; Rafiu (2012) reported 2.07, 0.12, 0.20, 0.61 and 0.22 % for N, P, K, Ca and Mg, respectively, while Tilander (1996) reported 2, 0.076 and 1.7% for N, P and K, respectively. All these studies indicate appreciable levels of N in Neem leaves although it is not a leguminous tree. Our results, show relatively higher levels of Ca (2.58 %) compared to those reported by others. In comparing the composition of neem leaves to other sources of aqueous leaf extract such as confrey (*Symphytum officinale*) and tithonia (*Tithonia diversifolia*), neem generally contains lower amounts of P and K (Malama, 2001).The results show that neem leaves make a good source of nutrients for the preparation of aqueous neem leaf extract; the composition of leaves, however, depends on the age of the leaves with younger leaves having higher levels of nutrients (Rafiu, 2012).

While the composition of neem leaf extract depended on the amount of leaves used in the preparation, the amounts of nutrients in the extract were not related to the amounts observed in the leaves. For example, despite calcium being highest in the leaves compared to magnesium and potassium, the neem leaf extract contained more potassium and magnesium per unit volume compared to calcium at almost all concentrations. This could be attributed to differences in the solubility of these elements and their structural roles. Earlier reports in other plant species have indicated higher solubility of magnesium in water than calcium (Schmidt, MacDonald, & Kelly, 1974).

The results from this study show that in general, neem leaf extract did not make a good soil amendment; apart from calcium, there were no significant changes in other soil chemical characteristics. The increase in Ca levels due to neem leaf extract amendment has previously been reported by Solomon et al. (2008), Moyin-Jesu (2012) and Elnasikh et al. (2011). In the current study, calcium levels in the soil increased by 5 % from 8.1 to 8.5 cmol+/kg. This increase in calcium levels did not, however, elicit an increase in pH as had been previously observed by Solomon et al. (2008). This could be attributed to the marginal increase in Ca and a lack of accompanying change in the levels of the other bases. This increase in calcium could be a beneficial result if the amendment is to soils with critically low calcium levels (below 0.1 cmol+/kg). Other studies have shown increases not only in calcium but also in organic matter, available phosphorus, total nitrogen, ammonium nitrogen, potassium, magnesium, pH, and micronutrients such as iron, manganese, copper and zinc due to amendment with neem (Gougoulias et al., 2012; Moyin-Jesu, 2012; Solomon et al., 2008).

The results of this study show some stimulatory effect of neem leaf extract application from 2 to 10 % concentration on microbial activity followed by a decline from 10 to 20 %, depicted in Figure 3. The increase in microbial activity from the control to 10 % concentration ranged from 13 to 21 and 2 to 12 % at weeks 5 and 10, respectively. This increase in activity could be attributed to the addition of mineralizable organic materials in the leaf extract. While this study did not report any significant change in most nutrients, addition of nutrients to the soil through neem leaf extracts has been implicated in observed increases in microbial activity (Solomon et al., 2008). The inhibitory effect of neem at 15 and 20 % could be attributed to the negative effects arising from the secondary metabolites associated with neem. Specifically, alkaloids, glycosides, flavonoids and saponins found in the leaf extract of neem have been shown to have anti-microbial activities (Maragathavalli, Brindha, Kaviyarasi, Annadurai, & Gangwar, 2012; Prashanth & Krishnaiah, 2014; Sarawaneeyaruk et al., 2015). At 15 and 20 %, the concentration of these anti-microbial metabolites could have increased to levels high enough to negatively impact microbial activity. It would have been best to determine the levels of these compounds in the leaf extract or soil in order to ascertain the relationship between microbial activity and the possible anti-microbial effects of the different neem leaf extract preparations.

Soil microbial biomass behaved differently from microbial activity with neem leaf extract addition to the soil. Figure 4 shows deviations (%) in microbial biomass from the control. The results suggest an initial depression followed by an increase and then a gentle decrease in soil microbial biomass. This behavior was previously reported by Solomon et al. (2008). The most microbial biomass and therefore, largest positive deviation from the control was seen at 15 % concentration of neem leaf extract. As previously indicated, some of the increase could be attributed to the addition of mineralizable nutrients in the extract. A shift in the population of microbes with neem leaf extract amendment has been suggested to favor some microorganisms over others (Solomon et al., 2008; Sayed et al., 2011). So the observed increase in microbial biomass between 5 and 15 % could be explained by an increase in the population of those organisms able to utilize mineralizable components added in the neem leaf extract. Carbon degraders such as *Bacillus, Arthrobacter*, and *Pseudomonas* species have been shown to be selectively favored with the addition of neem products to the soil (Solomon et al., 2008). In general, application of neem products to the soil has been shown to reduce the growth of bacterial and fungal strains at high concentrations (Gougoulias et al., 2012; Maragathavalli et al., 2012; Sayed et al., 2011).

Though the results for soil organic matter content did not differ significantly among the different neem preparations, the highest soil organic matter was recorded at 15 % neem leaf extract concentration. These results corroborate with already established relationships between soil organic matter content and microbial biomass; soil microbial biomass is known to highly correlate with soil organic matter content (Sarawaneeyaruk et al., 2015). It is difficult to determine the relationship between calcium and soil microbial biomass in this study although calcium has been shown to be particularly important for bacterial proliferation.

In conclusion, although the application of neem leaf extract at 10 % percent or higher can inhibit both microbial biomass and activity, some mineralizable components in the extracts can support growth and activity of some microorganisms in the soil. While amending the soil with neem leaf extract did not particularly improve selected chemical attributes of the soil in this study, it increased soil Ca levels. The use of neem leaf extract can therefore be of benefit to soils with critically low levels of Ca.

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