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Assessing The Bioconcentration of Heavy Metals in Nypa Palm (*Nypa fruticans* (Wurmb) From Selected Mangrove Forests in Rivers State, Nigeria

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ABSTRACT: The bioconcentration of Pb, Zn, Cd, Cr and Ni in tissues of Nypa palm (*Nypa fruticans*) was investigated during wet and dry seasons from selected mangrove forests, using the randomized complete block design. Soil and plant samples were randomly collected from plant dominated growth stations and analyzed for heavy metal content using the Perkin Elmer Analysts 200 Atomic Absorption Spectrophotometer (AAS). The results obtained showed the bio-transfer factors of heavy metals at wet season as Pb (1.08mg/kg), Zn (1.42 mg/kg), Cd (6.14 mg/kg), Cr (0.56 mg/kg) Ni (0.90 mg/kg) and dry season as Pb (0.86 mg/kg), Zn (1.15 mg/kg), Cd (5.44 mg/kg), Cr (0.66 mg/kg), Ni (1.12 mg/kg). The result further showed the bio-translocation factors of the metals at wet seasons as Pb (0.59 mg/kg), Zn (0.76 mg/kg), Cd (3.25 mg/kg), Cr (0.22 mg/kg), Ni (0.54 mg/kg) and dry season as Pb (0.64 mg/kg), Zn (1.03 mg/kg) Cd (3.07 mg/kg), Cr (0.24 mg/kg), Ni (0.61 mg/kg) respectively. Findings indicate the study plant as a hyper accumulator of Ni, Cd and Zn, and non-hyper accumulator of Cr and Pb. It is thus recommended that *N. fruticans* be utilized for phytoremediation of Ni, Cd and Zn in polluted mangrove ecosystem.

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Key words: Heavy metal, Bioconcentration, Accumulation, Phytoremediation, Bio-transfer factor, Bio-translocation factor, Mangrove.

Globally, *Nypa fruticans* is identified as an integral component of the Asian mangrove forest whose distribution cuts across Europe, Africa and America (Duke, 2006; Dransfield *et al*., 2008). The plant was introduced to Cameroon and Nigeria in the African sub region from the Singapore Botanical Gardens in South East Asia in 1906 (Tuley,1995), from where it spread and naturalized in Panama and Trinidad, South America (Dransfield *et al*., 2008). The introduction to Nigeria was for the purpose of stabilizing the coastline against erosion (Udoidiong and Ekwu, 2011). This mangrove species after establishment has spread west ward across the region to Ondo State, invading expands of land, displacing the valuable endemic mangrove species. The trend of displacement cuts across the Niger Delta of Nigeria and Cameroon forming mono specific stands that compete against the native mangrove species (Saenger *et al*., 1995). The adaptation of the plant to its environment is due to the possession of underground prostrate rhizomes that branch dichotomously and from which young plants develop by vegetative propagation, thus forming extensive stands that are closely packed together (Teo *et al*., 2010). They also possess root systems that are extensive and capable of resisting swift water current (Percival and Womersley, 1979). *Nypa fruticans* strive well in calm mangrove ecosystem with high inflow of fresh water (Tomlinson, 1986), and where sediments from the sea are deposited forming clay soil with brackish water and anaerobic condition (Rozainah and Aslezaeim, 2010). They propagate by means of viviparous germination (Tomlinson, 1986), while the seeds are dispersed and deposited inlands as far as the tide can reach. Niger Delta of Nigeria is noted for the production of light crude, with associated adverse negative impacts on mangrove forest (Duke *et al*., 2000). The nature of mangrove forest devastation from anthropogenic activities has resulted to its classification as one of the forests with extremely rapid rate of depletion (FAO, 2005). Beside environmental impact of crude oil, other sources of heavy metals include discharges from industrial and municipal waste waters, storm, run-off, dust deposition, mine discharge and waste incineration (Cheng *et al*., 2009; Besser *et al*., 2009). Heavy metals and sewage constitute a major threat to mangrove forest (Agoramoorthy *et al*., 2008), and the understanding of varying concentrations of heavy metals, the nature of distribution coupled with their relative toxicity and persistence in the environment

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has bestowed it as a priority pollutant for environmental management globally (Don- Pedro *et al*., 2001). Mangrove soils have been shown to possess high capacity of retaining heavy metals which are taken up by plants (Machando *et al*., 2002). Studies have also shown that wetland plants possess the capacity for accumulation of heavy metals in their tissues (Yadav and Chandra, 2011). Such plants accumulate high levels of pollutants in their tissues above that of the contaminated media (Erakhrumen, 2014). Bert *et al*. (2003) identified three categories of plants that grow in contaminated sites as tolerant, indicator and hyper accumulators. While tolerant plants successfully grow in environment with high concentrations of pollutants that poison other plants, hyper accumulator plants take up these pollutants and store them in various tissues without harmful effect on their growth. Indicator plants grow and subsist in the environment with high concentrations of pollutants. Studies have shown that *Nypa fruticans* possesses high tolerance for Cr and Zn toxicity than *Rhizophora racemosa* (Gbosidom, *et al*., 2017). In their study; it was shown that Zn levels significantly influenced the growth and distribution of *Nypa fruticans.* Plants which have the tolerance and capacity to take up trace elements from contaminated soils are being used for phytoremediation (Mery *et al*., 2011). Nirmal *et al*. (2011) in their study on the assessment of the accumulation potential of Pb, Zn and Cd by *Avicinnia marina* in Vamleshwar mangrove of Gujarat in India, reported that *A. marina* possessed the capacity to take up Zn and Cd through their roots, accumulating them in the leaves without showing any sign of injury. It was also observed that the roots of the plant accumulated high concentration of the metals with the exception of Cd. Also, in studying the accumulation and partitioning of As, Cd, Cr, Cu, Hg, Pb and Zn in the three estuarine wetlands in the Hainan Island of China, Qiu *et al*. (2011) reported that Pb was found in the branches of the different mangroves studied. Apart from the use of hyper accumulator plants to extract heavy metals from the environment, non-hyper accumulators accumulate higher concentrations of heavy metals in their roots than the shoots (McGrath *et al*., 2001). In a bid to solve the environmental problems associated with heavy metals in mangrove forest through phytoremediation, this study was designed to evaluate the bio concentration of heavy metals in *Nypa fruticans* and its application in phytoremediation.

MATERIALS AND METHODS

Study Area: The study area stretches from Longitude $7^0 05' 00'' E$ through Longitude $7^0 30' 30'' E$ and latitude $4^0 45' 30'' N$, encompassing communities such as Kono (station 1), Bomu (station 2), Ogu (station 3) and Port Harcourt (station 4). These are the areas enlisted for the study.

Experimental Design: The randomized complete block design (RCBD) method consisting of 4 stations was used for the study.

Field Sampling: Field sampling was done in two seasons. The wet season sampling was undertaken from July to August 2015, while dry season sampling was done from January to February, 2016.

Soil and Plant Sampling: Soil samples were collected randomly at 0 - 30 cm closer to the roots of *Nypa fruticans* at their dominant growth area in three replications each using soil auger. The above-ground (Leaves and stems) and below-ground (roots) samples of *N. fruticans* were randomly collected from the four study stations in three replications each, using sharp knife and hoe. Soil and plant samples were stored in sterile cellophane bags, labeled with sample codes for easy identification and transported in plastic coolers to the laboratory for the evaluation of heavy metals.

Sample Preparation: Soil and plant samples were prepared at the Chemistry Laboratory of Science Laboratory Technology in Ken Saro-Wiwa Polytechnic, Bori. Soil samples were dried in the hot air oven (model T 5028) at 100^0 C and crushed into powdered form using porcelain mortar and pestle, after which samples were sieved using skitter. The prepared samples were then stored in clean cellophane bags and labeled according to their respective to sample identity.

The above ground tissues (leaves/stem) and the below ground tissues (root) samples were dried in the oven at 150^0 C, as separate samples using porcelain mortar and pestle. Pulverized samples were stored in clean cellophane bags and labeled according to sample identity.

Digestion of Samples: Pulverized soil samples were digested by weighing out 1g each, using analytical weighing balance (model TH1000) into respective Pyrex beakers and labeled according to sample identity. To each sample, 5ml HNO_3 (70% v/v) was added, followed by 5ml $HCLO_4$ (70% v/v) and then 10ml HF (48% v/v). The resultant solution was subjected to heating, using the heating mantle (model MY 6403) until the sample was dry. The residue was allowed to cool. After cooling, the residue was dissolved in 5ml HCL (36% v/v) and 20ml distilled water after which the digested samples were filtered, washed using cotton wool into different prelabeled 100ml volumetric flask. The digested samples were

made up to 100ml by addition of distilled water. The above procedure enhanced the dissolution of the soil silicate matrix consequently yielding total digestion. The digested samples were stored in sample bottles and labeled for heavy metal analysis.

Plant samples were digested by weighing 2g of the pulverized samples, using analytical balance (model-TH 1000) into 250ml beakers. To each beaker containing samples, 132ml of distilled water was added. Also, 5ml HNO_3, 10ml of H_2SO_4 and 2ml H_2O_2 were added and made up to 150ml using distilled water. The resultant mixture was heated using heating apparatus in a fume cupboard till the volume was reduced to about 50ml.The digested sample was allowed to cool, then filtered and washed using cotton wool into a 100ml volumetric flask and made up to 100ml. Samples were stored in sample bottles and labeled according to samples identity for heavy metal analysis.

Heavy Metal Analysis: Heavy metal content of soil and plant tissues were determined using the Perkin Elmer Analysts 200 Atomic Absorption Spectrophotometer (AAS). Cr, Nickel, Cd, Pb and Zn were determined based on their respective wave lengths. A certified standard stock solution of 1000ppm was prepared for the analysis, from which working standards were prepared based on the linearity range of each metal. Compressed air was used as oxidant while acetylene gas was used as fuel gas. To set zero absorbance, distilled water was used as a blank while the instrument was optimized based on the characteristics of the respective metals to be determined. Calibration curves were prepared using 4 to 5 working standards for each metal. The concentration of samples was read by the AAS in ppm and printed.

Determination of Bio Transfer and Translocation Factor (TF): Bio transfer factor of pollutants were quantified according to Kumar *et al.*, (1995) method using the formula

$$\text{Bio transfer factor (BTF)} = \frac{C_{biota}}{C_{soil}}, \qquad (1)$$

Where C_{biota} is the total concentration of metal or pollutant in biota, while C_{soil} is the total concentration of metal or pollutant in the soil.

The translocation factor (TF) of pollutants in plant samples were estimated using the equation (2) by (Barman *et al.*, 2000; Gupta *et al.*, 2008):

$$\text{TF} = \frac{Concentration\ of\ pollutants\ in\ shoot}{Concentration\ of\ pollutants\ in\ root} \qquad (2)$$

RESULTS AND DISCUSSION

*Bio Transfer Factor of Heavy Metals***:** The result of wet season means bio transfer factor (BTF) of Cr in *N. fruticans* tissues presents a trend of station 4 > station 1 > station 3 > station 2. The mean TF for the four stations was 0.56 mg/kg. These results did not indicate statistical differences between the mean TF of Cr at the study stations at p = 0.05 (Figure 1). The dry season mean transfer factor (BTF) of Cr in *N. fruticans* tissues was observed with a trend showing that station 4 > station 1 > station 3 > station 2, with the mean TF of the study stations as 0.66 mg/kg. The above results were not statistically different between the stations at p = 0.05 (Figure 1). The result of wet season transfer factor of Cd in *N. fruticans* at the study stations indicated the trend of station 2 > station 1 > station 3 > station 4, while the mean transfer factors of the four study stations showed the value of 6.14 mg/kg. The above result had no statistical differences in transfer factors of Cd at p = 0.05 (Figure 2). At dry season, the trend for mean transfer factor is station 3 > station 4 > station 2 > station 1, with a mean transfer factor of 5.44 mg/kg for the four study stations. There were no statistical differences in bio transfer factors at p = 0.05 (Figure 2). The result of wet season means bio transfer factor (BTF) of Ni in *N. fruticans* growth soil and plant showed the trend of station 3 > station 1 > station 4 > station 2. The result further showed the mean transfer factor of the four study stations as 0.90 mg/kg. From these results there is statistical difference between stations 1 and 3, 2 and 3, and between station 3 and 4 at p = 0.05 (Figure 3). At dry season, mean transfer factor of Ni showed the trend of station 1 > station 3 > station 4 > station 2, with the four study stations showing a mean transfer factor of 1.12 mg/kg. Furthermore, there is statistical differences between stations 1 and 2, 1 and 4, and between station 2 and 3 at p = 0.05 respectively (Figure 3). The result of wet season mean bio transfer factor (BTF) of Pb in *N. fruticans* tissues is observed with a trend of station 3 > station 4 > station 1 > station 2. The mean TF of Pb of the four study stations as 1.08mg/kg. These results showed no statistical differences in mean transfer factor of Pb at p = 0.05 (Figure 4). The dry season results produced the mean transfer factor of trend of station 4 > station 3 > station 1 > station 2, with a TF of 0.86 mg/kg for the study stations. These results displayed statistical differences in mean transfer factors of Pb between the stations at p = 0.05. The LSD result further showed statistical difference between stations 2 and 3 and between station 2 and 4 at p = 0.05 respectively (Figure 4). The wet season mean transfer factor of Zn in *N. fruticans* growth soil and plant tissues exhibits the trend showing that station 4 > station 3 > station 2 > station 1. The mean transfer factor of Zn of the four study stations as 1.42mg/kg.

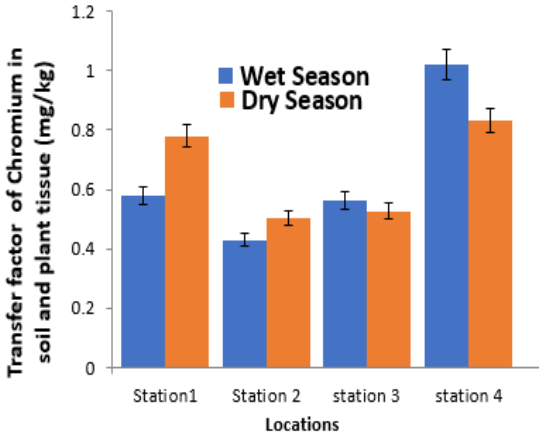

Fig. 1: Bio Transfer Factor of Cr in *N. fruticans*

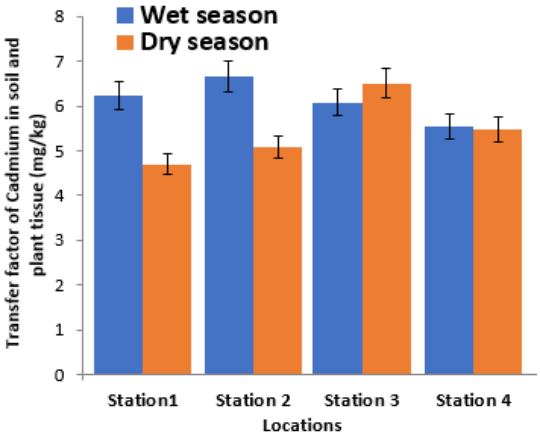

Fig. 2: Bio Transfer Factor of Cd *N. fruticans*

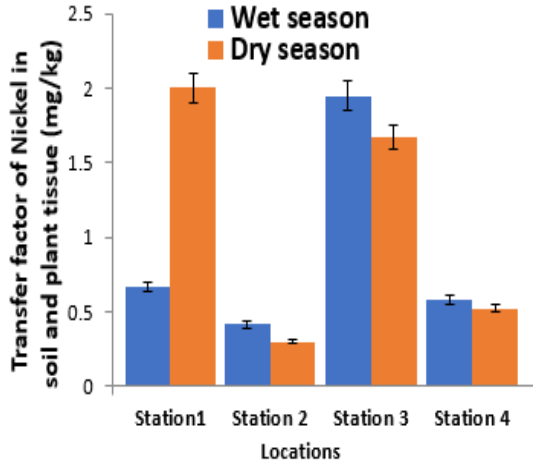

Fig. 3: Bio Transfer Factor of Ni in *N. fruticans*

There were no significant differences in transfer factors based on stations at p = 0.05 (Figure 5). At dry season, the results were observed with a trend showing that TF of station 3 > station 4 > station 2 > station 1, with the mean transfer factor of Zn for the four study stations 1.15 mg/kg. These results were statistically different based on stations at p = 0.05. The LSD result on the mean transfer factor of Zn showed statistical differences between stations 1 and 3, 1 and 4, 2 and 3, and between station 2 and 4 at p = 0.05 respectively (Figure 5).

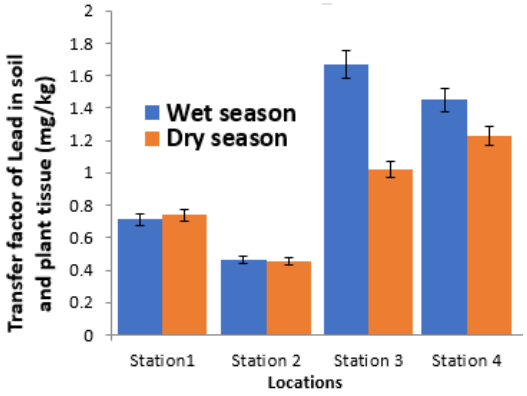

Fig. 4: Bio Transfer Factor of Pb in *N. fruticans*

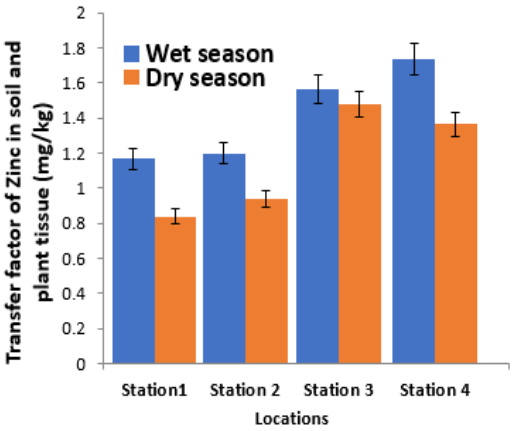

Fig. 5: Bio Transfer Factor of Zn in *N. fruticans*

Translocation Factor Of Pollutants: The wet season result of mean translocation factor (TF) of Cr in the root and shoot tissues of *N. fruticans* showed the trend of station 2 > station 3 > station 1 > station 4. The result also showed the mean translocation factor for the four study stations as 0.22 mg/kg. The above result indicated statistical differences in TF of Cr in *N. fruticans* based on stations at p = 0.05. The LSD results showed statistical differences in Cr TF between stations 1 and 2, 2 and 3, and between station 2 and 4 at p = 0.05 respectively (Figure 6). At dry season, the mean translocation factor of Cr in root and shoot tissues of *N. fruticans* exhibited the trend of station 2 > station 1 > station 3 > station 4. The above result also showed the mean translocation factor of the four study stations as 0.24 mg/kg. These results were statistically different based on stations at p = 0.05. The LSD result further showed difference in Cr TF between stations 1 and 2, 2 and 3, and between station 2 and 4 at p = 0.05 respectively (Figure 6).

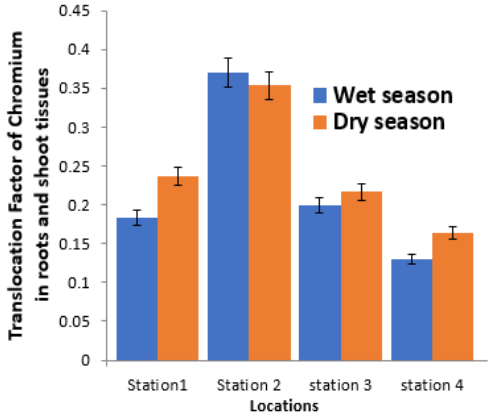

Fig. 6: Translocation Factor of Cr in *N. fruticans*

The study on wet season translocation factor of Cd in *N. fruticans* root and shoot was observed as station 1 > station 2 > station 4 > station 3. The mean translocation factor of the above four study stations was observed as 3.25 mg/kg. These results were not statistically significant in terms of difference based on stations at p = 0.05 (Figure. 7). The dry season study followed the trend of station 1 > station 2 > station 4 > station 3 in translocation factor of Cd in roots and shoots of *N. fruticans*. The results of mean translocation factor of the four study stations was observed as 3.07 mg/kg. There were observed statistical differences between the mean Cd translocation factors based on stations at p = 0.05. The LSD result further indicated statistical differences between stations 1 and 3, 2 and 3 and between station 3 and 4 at p = 0.05 (Figure. 7).

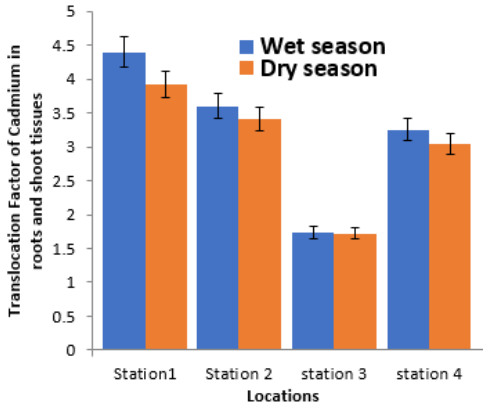

Fig. 7: Translocation Factor of Cd in *N. fruticans*

The result of wet season translocation factor of Ni in the root and shoot tissues of *N. fruticans* specified the trend of station 2 > station 3 > station 1 > station 4. The result also indicates the mean translocation factor of the four study stations as 0.54 mg/kg. Furthermore, there were statistical differences in translocation factors based on stations at p = 0.05. The LSD result clearly indicate statistical difference between stations 1 and 2, 1 and 3, 2 and 3, 2 and 4, station 3 and 4 (Figure 8). The dry season study the displayed the trend of station 3 > station 2 > station 1 > station 4 in translocation factor of Ni, while the mean translocation factor of the four study stations amounted to 0.61 mg/kg. Furthermore, these results were not statistically different at p = 0.05 (Figure 8).

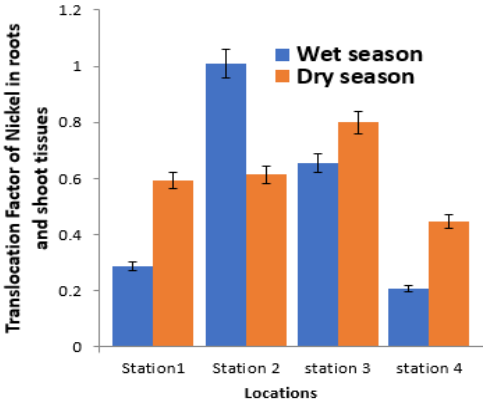

Fig. 8: Translocation Factor of Ni in *N. fruticans*

The wet season result of mean translocation factor (TF) of Pb in *N. fruticans* root and shoot tissues indicates a trend showing station 2 > station 1 > station 4 > station 3. It was further observed that the mean TF of Pb at the four study stations was 0.59 mg/kg. There were also statistical differences in mean translocation factor of Pb based on stations at p = 0.05. The LSD result further validate differences in mean translocation factor of Pb between stations 1 and 2, 1 and 3, 2 and 3, and between station 2 and 4 at p = 0.05 respectively (Figure 9). During the dry season, the result of mean translocation factor of Pb in *N. fruticans* root and shoot tissues was observed to indicate a trend of station 2 > station 1 > station 4 > station 3, while the mean translocation factor of the study stations was 0.64 mg/kg. These results displayed statistical differences in mean translocation factor of Pb in *N. fruticans* root and shoot tissues at p = 0.05. The LSD result further indicate differences in mean translocation factor of Pb between stations 1 and 2, 1 and 3, 2 and 3, 2 and 4, and between station 3 and 4 at p = 0.05 respectively (Figure 9). The result of wet season study on translocation factor of Zn in root and shoot tissues of *N. fruticans* followed the trend of station 1 > station 2 > station 4 > station 3, while the mean translocation factor of the four study stations was observed as 0.76 mg/kg. The above results were not statistical different based on stations at p = 0.05 (Figure. 10). The result of dry season study indicated a trend showing translocation factor of station 1 > station 2 > station 3 > station 4. The mean translocation factor of the four study stations was 1.03 mg/kg. This result was not statistical different based on stations at p = 0.05 (Figure. 10).

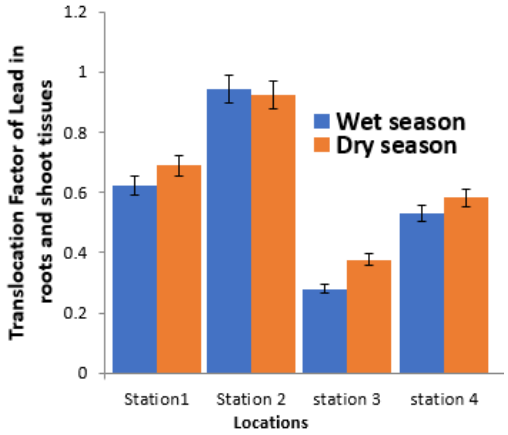

Fig. 9: Translocation Factor of Pb in *N. fruticans*

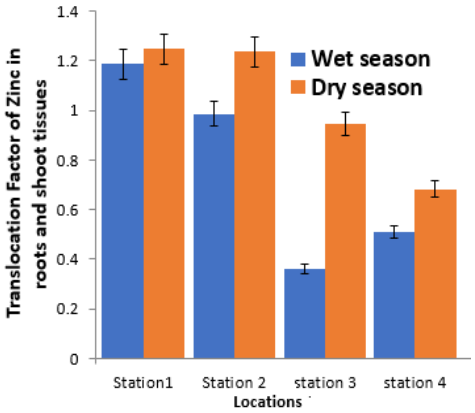

Fig. 10: Translocation Factor of Zn in *N. fruticans*

The study on comparison of pollutant accumulation in the tissues of *N. fruticans* exhibited different reactions. Results obtained from Cr uptake indicates that *N. fruticans* accumulated more Cr in root tissues than shoot tissues. The result further expressed significant difference in root tissues across study stations. There were also significant differences in shoot tissues across stations. The observed high concentration of Cr in the root tissues could be attributed to the low mobility and bioavailability of Cr to natural phyto-extraction process as observed by Komerek *et al*., (2007). The results of Cr transfer factor (TF) did not show any statistical significance. However, the translocation factors were significant at 0.05 alpha levels with variability in least significant differences across the study stations. The results further indicated that the transfer and translocation factors were < 1.0, an indication that Cr was mainly concentrated in the ground (root) tissues of the plant. These findings corroborate the report of McGrath *et al*. (2001) who showed that some plants accumulated higher concentrations of heavy metals in their roots than shoot tissues and thus referred to them as non-hyper accumulators. It can thus be inferred that *N. fruticans* possessed higher potentials for chromium accumulation in their root tissues. Similarly, Debargha *et al*. (2013) in their study observed low bioaccumulation factor for Zn, Cu, Pb and Cr in *Avicennia officinalis* with TF < 1, an observation which they interpreted as an indication that *A. officinalis* took up and translocate the above metals below the levels exhibited by hyper accumulators. The results obtained on Ni accumulation showed that *N. fruticans* accumulated more concentrations of Ni in their below ground (roots) tissues than the above ground (shoot) tissues, with significant differences across the study stations at $p < 0.05$. The above finding contradicts the report of Pahalawattaarachchi *et al.* (2009) who observed that Nickel was concentrated in the shoots of *Rhizophora macronata* than in their roots. However, the finding was in tandem with those of Komerek *et al.* (2007) who observed high concentration of Cr in root tissues than shoot tissues. The findings supported the fact that plants species possess different accumulation potentials for different metals (Cheng, 2003). The study showed that the transfer factor of Ni in *N. fruticans* was > 1, a finding that qualify *N. fruticans* as a hyper accumulator of Nickel. Earlier reports showed that hyper accumulators are not only tolerant for high concentration of pollutants but also exhibit bio concentration factor and translocation factor > 1 (Ma *et al.*, 2001).

The results obtained on Cd accumulation showed that *N. fruticans* accumulated more concentrations of Cd in the above ground (shoot) tissues than the below ground (root) tissues. This result was significant across stations at $p < 0.05$. The above finding is in agreement with an earlier report by Pahalawattaarachchi *et al.* (2009) who observed high concentration of cadmium, zinc, Nickel and lead in the shoot of *Rhizophora macronata* than their roots. Similar observation was reported in *Avicennia marina* where cadmium was concentrated in leave tissues (Nirmal *et al.*, 2011). The evaluated transfer and translocation factors of Cadmium in *N. fruticans* was > 1.0. The above finding corroborates the report of Ma *et al.* (2001) who stated that plants that are tolerant to high concentration of pollutants exhibited bio concentration and translocation factors > 1. Similarly, Erakhrumen, (2014) classified heavy metal accumulating plants as those heaving concentrated pollutants in their tissues above that of the contaminated media. It is thus inferred that *N. fruticans* is a hyper accumulator of cadmium. Results on lead (Pb) accumulation in the tissues of *N. fruticans* showed higher accumulation in the below ground (root) tissues than the above ground (shoot) tissues. These concentrations were all significant at $p < 0.05$

across the study stations. The above observation is in line with the finding of Komerek *et al.* (2007) who reported the accumulation of heavy metals in root tissues and attributed it to the low mobility and bioavailability of such metals to natural uptake process by plants. Other corroborated reports on accumulation of metals in root tissues of plants include Pahalawattaarachchi *at al.,* (2009) and Almasheer *et al.,* (2014). The transfer factor of Pb in *N. frusticans* was observed to range between 0.86 and 1.08, with translocation factors between 0.59 and 0.64. Both transfer and translocation factors were significant at $p < 0.05$ across stations. Similar low bioaccumulation factor < 1 was reported in *Avicennia officinalis,* which was interpreted as an indication that *A. officinalis* take up Pb and translocate them below the levels exhibited by hyper accumulators. It can thus be inferred that *N. fruticans* is a non-hyper accumulator of Pb. Evaluation of zinc (Zn) accumulation in the tissues of *N. fruticans* showed that *N. fruticans* as an accumulator of Zn in the below ground (root) tissues, with a transfer factor > 1.0. This result was significant across stations at $p < 0.05$. The result of the translocation factor was > 1.0, however the result was not statically significant across stations. The above findings qualify *N. fruticans* root as a non-hyper accumulator of Zn, which is in line with McGrath *et al.* (2001) who reported that non-hyper accumulator plants accumulate higher concentrations of heavy metals in their roots than the shoots. Root tissues have also been shown to accumulate higher concentrations of most metals than the above ground tissues (Pahalawattaarachchi *et al*., (2009) and Almasheer *et al.*, (2014). The high accumulation of metals in root tissues was associated with low mobility and bioavailability of the metals to natural phytoextraction process (Komerek *et al*., 2007).

Conclusion: The bioconcentration of heavy metals in *N. fruticans* was investigated in this study. Findings consequently established the study plant as a non-hyper accumulator of Cr and Pb in root tissues, and hyper accumulator of Zn and Ni in root tissues and Cd in shoot tissues. It is therefore recommended that *N. fruticans* can be used for the phytoremediation of Ni, Cd and Zn for which they show good potential for accumulation.

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