

Evaluation of Heavy Metal Phytoextraction Potential of *Rhizophora Racemosa* in Niger Delta Mangrove Forest, Rivers State, Nigeria

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Abstract

The bio transfer and bio translocation factors of heavy metals in the tissues of *Rhizophora racemose* was evaluated in this study. Soil, roots and shoot samples of the study plant were randomly collected from Kono, Bomu, Ogu and Borokiri mangrove forests at wet and dry seasons, digested and analyzed for heavy metals using standard laboratory methods. The laboratory results of field samples were further subjected to bio transfer and bio translocation factors analysis. Findings on wet season bio transfer factor revealed the concentrations of Cr (0.36mg/kg), Ni (0.86 mg/kg), Cd (2.86 mg/kg), Pb (1.17 mg/kg) and Zn (1.85 mg/kg), while dry season showed Cr (0.36mg/kg), Ni (0.55 mg/kg), Cd (1.24 mg/kg), Pb (2.72 mg/kg) and Zn (1.99 mg/kg). The bio translocation factor results for wet season indicated the concentrations of Cr (0.51mg/kg), Ni (1.47 mg/kg), Cd (1.02 mg/kg), Pb (1.48 mg/kg) and Zn (0.88 mg/kg), while dry season result revealed Cr (0.41mg/kg), Ni (0.65 mg/kg), Cd (1.24 mg/kg), Pb (1.50 mg/kg) and Zn (0.81 mg/kg). This study therefore classifies *Rhizophora racemose* as a hyper accumulator of Pb and Cd in shoot tissues and non-hyper accumulator of Cr, Ni and Zn in root tissues. Beside other uses, the plant has shown high affinity for the accumulation of heavy metals and thus can be used for phytoremediation.

Keywords: Phytoextraction, mangrove, heavy metals, evaluation, bio-transfer, bio-translocation

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1. Introduction

The Niger Delta region has over the years been prone to various forms of pollution, some of which are due to inundation by oil spills. Oil spillage in the region had been attributed to pipeline corrosion, leakages from well heads, poor maintenance of oil related infrastructures, human errors, oil theft and vandalization (Adeola, 2000; Amnesty International 2009).

The pollution of this coastal belt is further aggravated by poor regulation of oil related activities such as exploration and exploitation of crude oil and gas (Erakrumen, 2007). The Niger Delta mangrove swamp forest had been reported as highly productive for fisheries resources (Akankali and Jamabo, 2012). This mangrove forest falls among locations that are exposed to large deposits of wastes and pollutants due to the innate characteristics of growing in environments with tidal fluctuation (Erakrumen, 2014). Studies have shown that about 35% of endangered red and threatened species are either located or depend on wetland habitats and fish species require the estuaries either as nursery ground or for their entire lives' subsistence (Sulton and Shun, 1996).

Information on heavy metals pollution is necessitated as it is currently a problem needing global attention (Marchand et al, 2006), consequent on the premise that they are non-biodegradable and their persistence and accumulation in the environment causes toxicity in organisms (Ghosh and Singh 2005, Neff et al., 2006).

The sources of heavy metals are both natural and anthropogenic while natural sources contribute lesser proportion, anthropogenic sources contribute greater percentage. These sources include but not limited to mining and smelting, industrial waste, pipeline vandalization, domestic waste, fertilizers, pesticides, sewage sludge. and oil spill. The 2004 World congress on environmental health observed that metal poisoning of the environment is becoming a major public health problem in African Countries due to industrialization and globalization (Carnie, 2004).

In a bid to revegetate highly contaminated sites by heavy metals, metal tolerant plant species are cultivated to cushion the effect of migration of contaminants by soil leaching, contamination of ground water, wind and transportation of exposed soil surfaces (Stoltz and Greger, 2002; Tordoff et al, 2000). These plants possess the characteristics that enable them to absorb metals by their roots and translocate them to shoot organs where they are accumulated at higher concentrations (Baryla et al, 2001).

Therefore, this study examines the potential of *Rhizophora racemose* for phytoextraction of heavy metals.

2. Methodology

The methodology adopted for this work is presented the subsections below.

2.1 Study Area

The study area for this research stretches from longitude 7° 05' 00" E through longitude 7°30' 30" E and latitude 4° 45' 30" N in Rivers State. Four stations in mangrove forests were chosen in four local Government Areas namely Kono (station 1) in Khana local government area, Bomu (station 2) in Gokana local government area, Ogu (station 3) in Ogu/gbobo local government area and Borokiri (station 4) in Port Harcourt City local government area, were enlisted and used for the study.

2.2 Sampling

The randomized complete block design (RCBD) was used in sampling. Wet season sampling was undertaken in the months of July and August, 2017, while dry season samples were collected in the months of January and February 2018. Soil samples were randomly collected at 0-30cm in three replications at the rhizosphere of study plant growth site using soil auger, and stored in clean cellophane bags. Plant samples (roots, leaf and stem) were randomly collected in three replicates each and stored in clean cellophane bags. All samples were labelled with appropriate sample identities, Samples were protected from external contaminants and were transported to the laboratory in plastic cooler containers for heavy metals analysis.

2.3 Laboratory Analysis

Plant samples were prepared by drying them to constant weight in hot air oven using model T5028 at 1500c. After drying, samples were pulverized into fine powder using mortar and pestle, while ensuring non-contamination. Pulverized samples were then sieved to enhance dissolution in solution during analysis.

Samples were digested by weighing 2g of each into respective 250ml beakers. To each beaker containing samples 132ml of deionized water was added. Subsequently, 5ml HN03, 10ml H2SO4 and 2ml H2O2 were added and made up to 150ml using deionized water.

The respective solutions were heated using heating apparatus in a fume cupboard till the volumes were reduced to about 50ml. The digested samples were allowed to cool after which they were filtered using cotton wool into 100ml volumetric flask and made up to 100ml by washing with deionized water. Samples were stored in pre-labeled sample bottles for heavy metal analysis.

Soil samples were dried at 1000c in a hot air oven (model T5028) to constant weight. Samples were then crushed, homogenized and sieved using skitter. Digestion was done following the method described by Eduardo et al, (2005). 1g of each sample was dissolved in 5ml HNO3 (70% v/v), 5ml HCL04 (70% v/v) and 10ml HF(48% v/v). The resultant solution was heated in a mantle (model MY6403) to dryness. The residue was allowed to cool, then dissolved in 5ml HCL (36% v/v) and 20ml deionized water. It was then filtered and washed through cotton wool into respective pre-labeled 100ml volumetric flasks, and made up to 100ml.

The concentrations of heavy metals in plant and soil samples were determined using Perkin -Elmer 200 Atomic Absorption spectrophotometer (AAS) in conformity with the described method by Perkin- Elmer corporation, (1996).

2.4 Determination of Bio Transfer Factor (BTF) and Bio Translocation Factor (BTLF)

The bio transfer factor (BTF) of heavy metals was evaluated according to Kumar *et al.*, (1995) method as stated below:

$$BTF = \frac{C_{biota}}{C_{soil}} \quad (1)$$

where C_{biota} is the total concentration of metal in plant, C_{soil} is the total concentration of metal in the soil.

Bio Translocation Factor (BTLF) of metal in plant samples were evaluated with the formula as in equation (2) (Barman *et al.*, 2000; Gupta *et al.*, 2008).

$$BTLF = \frac{\text{Concentration of metal in shoot}}{\text{Concentration of metal in root}} \quad (2)$$

3. Results

3.1 Bio transfer factor (BTF) of Cr in *R. racemosa*

The results of wet season mean bio transfer factor (BTF) of Cr in *R. racemosa* indicated the trend which showed that station 1 > station 2 > station 4 > station 3. The mean BTF for the four stations studied indicated 0.36 mg/kg of Cr concentration. Statistical analysis showed significant differences between the stations at $p = 0.05$. The result of least significant difference (LSD) for the above results further signified statistical differences between stations 1 and 2, 1 and 3, 1 and 4, 2 and 3, and between stations 2 and 4 at $p = 0.05$ respectively (Fig. 1). At dry season, the mean BTF of Cr similarly showed a trend of station 1 > station 2 > station 4 > station 3 levels. The result further showed the mean BTF of Cr concentration in *R. racemosa* for the four stations studies as 0.36

mg/kg. These results were statistically significant at $p = 0.05$. Furthermore, the LSD analysis indicated statistical differences between stations 1 and 2, 1 and 3, 1 and 4, and between stations 2 and 3 at $p = 0.05$ respectively (Figure 1).

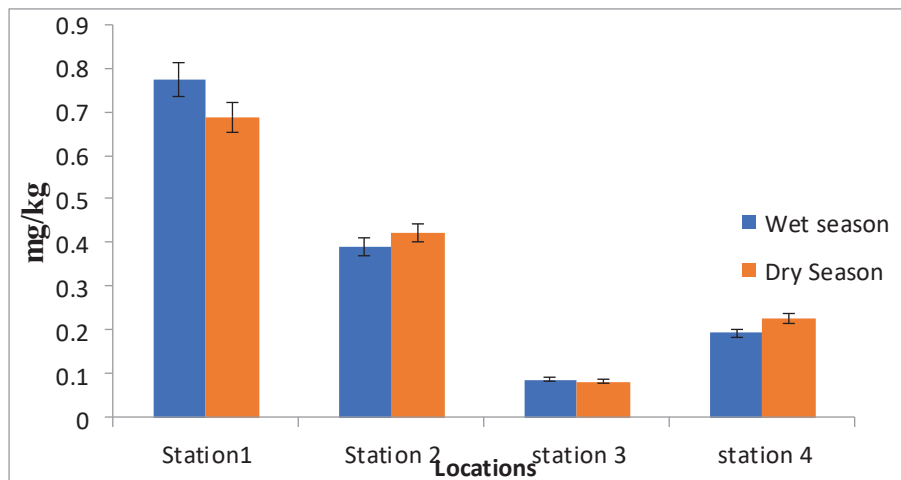


Figure. 1: Wet and dry season bio transfer factor of Chromium in *Rhizophora racemosa* soil and plant tissues.

3.2 Bio Translocation factor (BTLF) of Cr in *R. racemosa*

The result of wet season mean BTLF of Cr in the root and shoot tissues of *R. racemosa* presents the trend of station 4 > station 3 > station 2 > station 1 in concentration. These results further indicated the mean concentration of BTLF of the four stations studied 0.51mg/kg. However, the above results were not statistically different based on stations at $p = 0.05$ (Figure 2). The dry season result of mean BTLF of Cr in shoot tissues of *R. racemosa* revealed the trend of station 4 > station 3 > station 1 > station 2 in concentration, with mean BTLF of the four stations as 0.41mg/kg. These results were significantly different in BTLF at $p = 0.05$. Furthermore, the result showed differences in BTLF shoot tissues between stations 1 and 4, 2 and 3, and between station 2 and 4 at $p = 0.05$ respectively (Figure 2).

Figure 2: Wet and dry season bio translocation factor of Cr in shoots of *R. racemosa*

3.3 Bio transfer factor (BTF) of Ni in *R. racemosa*

The results obtained at wet season displayed the mean BTF of Ni in *R. racemose* with a trend that showed station 4 > station 3 > station 1 > station 2 in concentrations. The result also revealed the mean BTF of Ni in the four stations studied as 0.86 mg/kg. The above results were statistically significantly different at $p = 0.05$. The LSD result only indicated statistical differences between stations 1 and 2, 2 and 3, 1 and 4, and between stations 2 and 4 at $p = 0.05$ respectively (Figure 3). The dry season results unveiled a mean BTF trend of station 3 > station 1 > station 4 > station 2 in Ni concentrations, while the four stations studied displayed a mean concentration Ni BTF as 0.55 mg/kg. These results were significantly different at $p = 0.05$. The result further showed statistical differences in Ni BTF between stations 1 and 2, 2 and 3, 1 and 4, 2 and 4, and between stations 3 and 4 at $p = 0.05$ respectively (Figure 3).

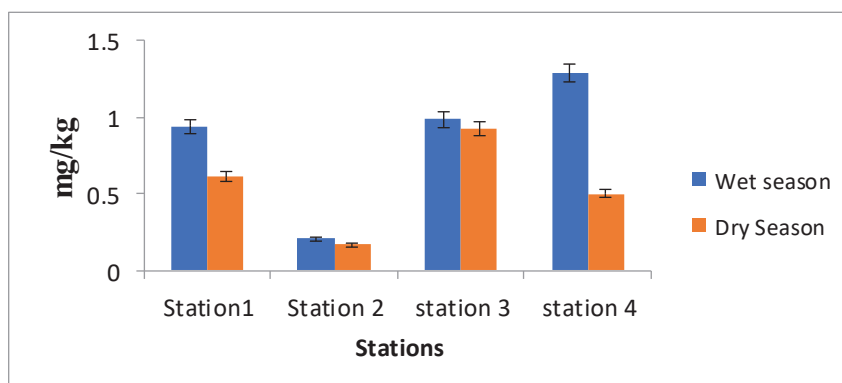


Figure 3: Wet and dry season bio transfer factor of Ni in *Rhizophora racemosa* soil and plant tissues.

3.4 Bio translocation factor (BTLF) of Ni in *R. racemosa*

The wet season result of mean BTLF of Ni in root and shoot tissues of *R. racemosa* presented a trend showing station 4 > station 2 > station 3 > station 1 in concentration. The result also indicated the mean BTLF of the four stations studied as 1.47 mg/kg. The result further displayed no statistical differences between BTLF of Ni in *R. racemosa* based on stations at $p = 0.05$ (Figure 4). The dry season result of mean BTLF of Ni in root and shoot tissues of *R. racemosa* showed the trend of station 4 > station 3 > station 2 > station 1 in concentration. The four stations studied unveiled a mean BTLF of 0.65 mg/kg. These results were not statistically significant at $p = 0.05$ (Figure 4).

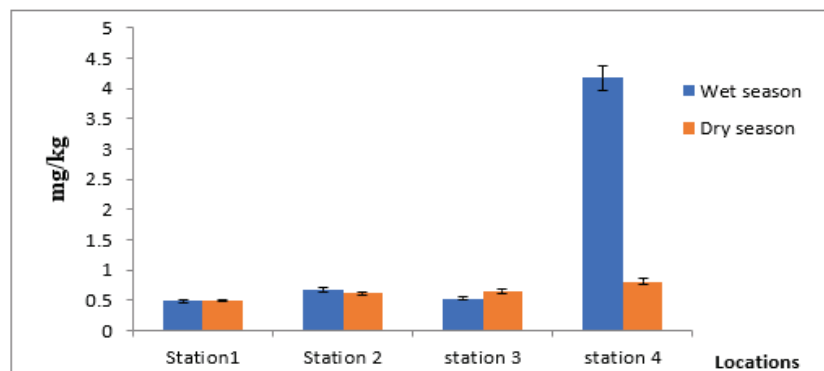


Figure 4: Wet and dry season Bio translocation factor of Nickel in *R. racemosa* root and shoot tissues

3.5 Bio transfer factor (BTF) of Cd in *R. racemosa*

The results of wet season mean bio transfer factor of Cd in *R. racemosa* revealed a trend showing station 3 > station 1 > station 4 > station 2 in concentration. The result further showed the bio transfer factor of the four study stations as 2.86. The above results were not significantly different based on stations at $p = 0.05$ (Figure 5). The result dry season result unveiled a trend of mean bio transfer factor concentration of station 3 > station 4 > station 1 > station 2, with a mean BTF of the four study stations as 1.24 mg/kg. These results were statistically significantly different based on stations at $p = 0.05$. The result also showed LSD between stations 1 and 3, 2 and 3, 3 and 4, and stations 2 and 2 at $p = 0.05$ (Figure 5).

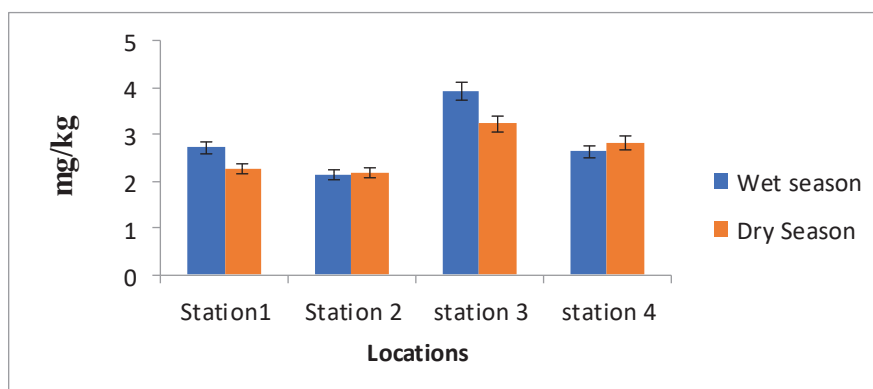


Figure 5: Wet and dry season bio transfer factor of Cd in *Rhizophora racemosa* soil and plant tissue

3.6. Bio translocation (BTLF) factor of Cd in *R. racemosa*

The result of wet season BTLF of Cd in *R. racemosa* root and shoot tissues showed the trend of station 4 > station 3 > station 2 > station 1 in concentration. The four stations studied had a mean BTLF concentration of 1.02 mg/kg. There were no significant differences in BTLF of Cd in *R. racemosa* root and shoot tissues based on stations at $p = 0.05$ (Figure 6). At dry season, the BTLF of Cd in *R. racemosa* root and shoot tissues indicated the trend showing station 3 > station 4 > station 1 > station 2 in concentration, with the mean of the four studied stations as 1.24 mg/kg. These results showed statistical differences in Cd BTLF based on stations at $p = 0.05$. The result further showed differences in Cd BTLF between stations 2 and 3 and between stations 2 and 4 at $p = 0.05$ respectively (Figure 6).

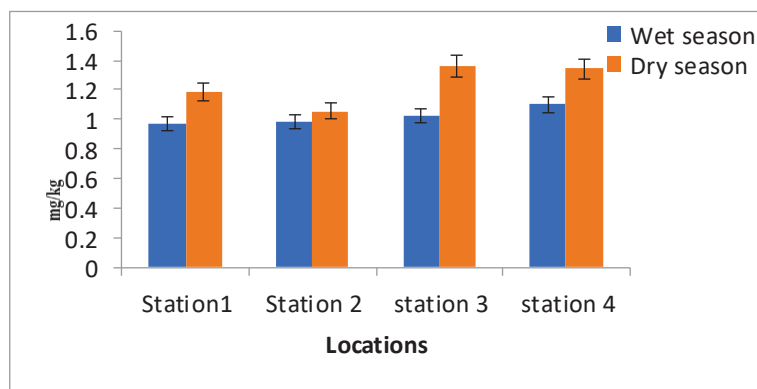


Figure 6: Wet and dry season bio translocation factor of Cd in *R. racemose* root and shoot tissues

3.7 Bio transfer factor (BTF) of Pb in *R. racemosa*

The result of wet season evaluation of mean BTF of Pb in *R. racemosa* soil and plant tissues showed the trend of station 1 >station 4 >station 2 >station 3 in concentrations. The result further indicated the mean BTF of Pb of the four study stations as 1.17 mg/kg. The result however displayed no statistical differences in the mean TF of Pb based on stations at $p = 0.05$ (Figure 7). At dry season, the mean BTF of Pb in *R. racemosa* soil and plant tissues showed the trend of station 2 >station 4 >station 1 >station 3 in concentration, the mean BTF of Pb for the four studied stations as 2.72 mg/kg. These results showed no statistical differences in BTF based on stations at $p = 0.05$ (Figure 7).

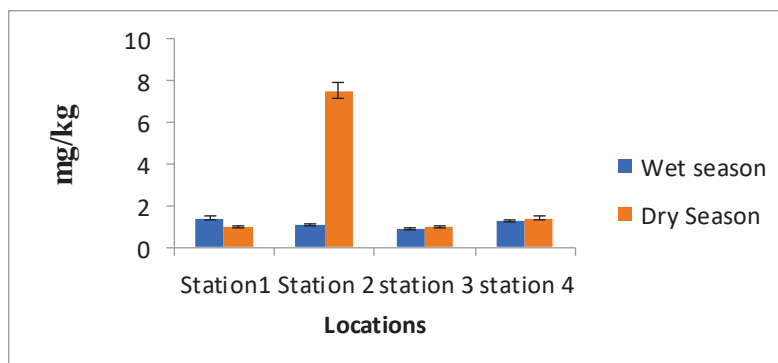


Figure 7: Transfer factor of Lead in *Rhizophora racemosa* growth soil and plant tissue

3.8 Bio translocation factor (BTLF) of Pb in *R. racemosa*

The wet season result of Pb BTLF in *R. racemosa* root and shoot showed a trend of station 2 >station 3 >station 4 >station 1 in concentration. The result further showed the mean level of Pb BTLF of the four stations studied as 1.48 mg/kg. The results were statistically significant based on stations at $p = 0.05$ (Figure 8). The dry season results presented the trend of station 2 >station 4 >station 3 >station 1 in Pb BTLF concentration. The result further showed the mean level of Pb BTLF of the four stations studied as 1.50 mg/kg. These results statistically significant based on stations at $p = 0.05$ (Figure 8).

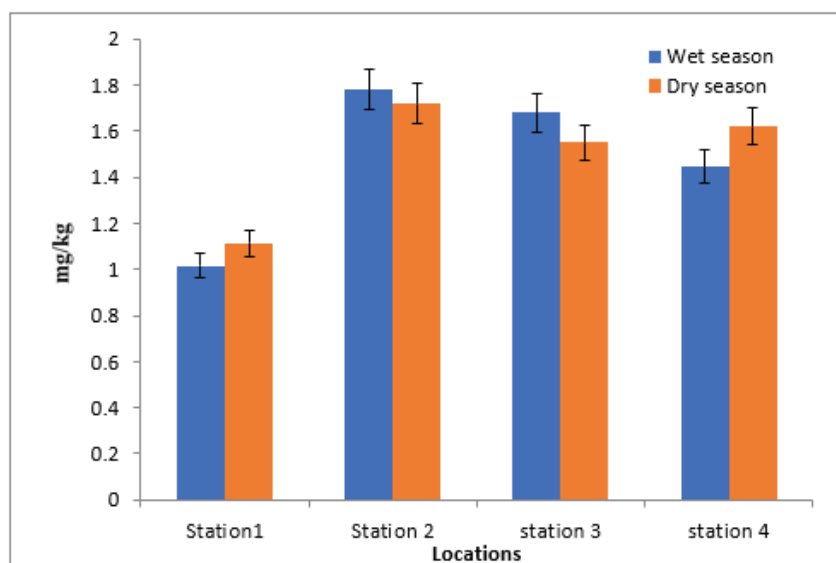


Figure 8: Wet and dry season bio translocation factor of Pb in R. racemose root and shoot tissues

3.9 Bio transfer factor (BTF) of Zn in R. racemosa

The result of wet season evaluation of Zn BTF in *R. racemosa* growth soil and plant tissues showed a trend of station 4 >station 2 >station 1 >station 3 in concentration. The result further revealed the Zn BTF of the four studied stations as 1.85 mg/kg. The results were not statistically different at $p = 0.05$ (Fig. 9). The dry season result revealed the Zn BTF trend of station 4 >station 2 >station 1 >station 3 in concentration. Furthermore, result showed the mean BTF of Zn at the four studied stations as 1.99 mg/kg. These results were not generally significantly different based on stations at $p = 0.05$. However, the LSD result indicated statistical differences between stations 1 and 2, 2 and 3, and between 3 and 4 at $p = 0.05$ (Figure 9).

3.10 Bio translocation factor (BTLF) of Zn in R. racemosa

The results of wet season BTLF of Zn in *R. racemosa* root and shoot tissues showed the trend of station 2 >station 3 >station 4 >station 1 in mean concentration. The result also showed the mean BTLF of the four studied stations as 0.88 mg/kg. These results showed no statistical differences in BTLF based on stations at $p = 0.05$. The LSD result only showed differences between stations 3 and 4 (Figure 10). The dry season results of presented a BTLF trend of station 4 >station 2 >station 3 >station 1 in mean concentration of Zn. The result further showed the mean BTLF of the four studied stations as 0.81 mg/kg. These results were not significant based on stations at $p = 0.05$ (Figure 10).

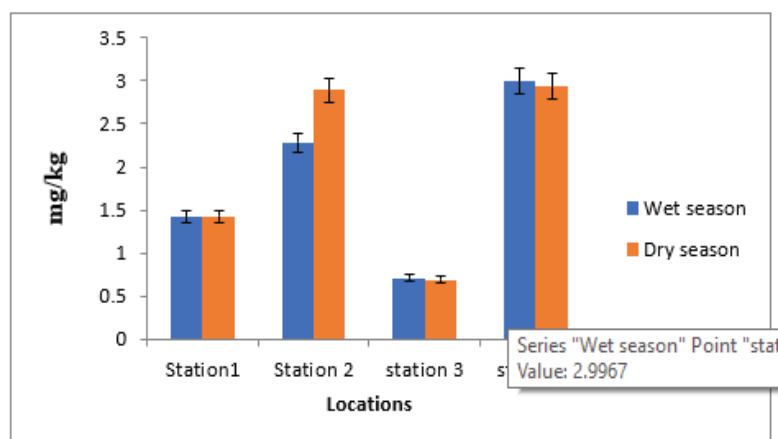


Figure 9: Wet and dry season bio transfer factors of Zn in *Rhizophora racemosa* soil and plant tissue

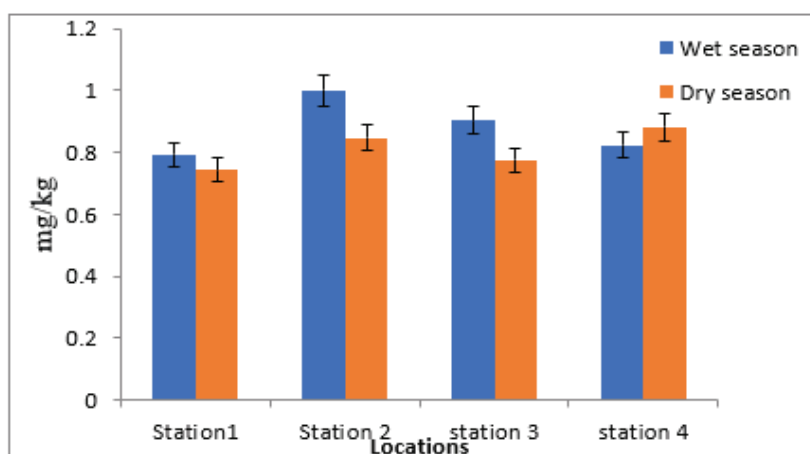


Figure 10:Wet and dry season bio translocation factors of Zinc in *R. racemose* root and shoot tissues

4 Discussion

This study on bio transfer and bio translocation factors of heavy metals in the tissues of *R. racemose* displayed different accumulation reactions with respect to the metal species studied.

Findings on Cr accumulation indicated that *R. racemosa* accumulated more Cr in their root tissues than their shoot tissues. The observed high concentration of Cr in the root tissues is attributable to bioavailability and low mobility of Cr to natural plant extraction process as observed by Komerek *et al.*, (2007). The result of Cr bio transfer factor (BTF) in *R. racemosa* was significant at $p < 0.05$, with significant LSD across stations. The result of bio translocation factor (BTLF) signified that higher concentrations of Cr was transferred into the roots of the plant and these BTLF levels were significant at $p = 0.05$, with variability in LSD across the study stations. The bio transfer and translocation factors were < 1.0 , an indication that Cr was mainly concentrated in the root tissues. The above findings corroborate the report of McGrath *et al.* (2001) who reported that some plants accumulated higher concentrations of heavy metals in their roots than shoot tissues and and consequently referred such plants as non-hyper accumulators. Similarly, Debargha *et al.* (2013) observed low bioaccumulation factor of Zn, Cu, Pb and Cr in *Avicennia officinalis* with BTLF < 1 , an observation which they interpreted as an indication that *A. officinalis* took up and translocated the referenced metals below the concentration exhibited by hyper accumulator plants. Also, Baker *et al.* (1994) described the concentration ratio of 1.0 as an indication that such metal was mainly accumulated in the shoots. It can thus be inferred from the findings of this study that *R. racemosa* is a non-hyper accumulator of Cr.

The results of Ni accumulation displayed that *R. racemosa* accumulated more concentrations of Ni in their roots than shoot tissues. These results were significant, with LSD across the study stations at $p < 0.05$. The above finding contradicts the report of Pahalawattarachchi *et al.* (2011) who observed that Ni were mostly concentrated in the shoot tissues than root tissues of *Rhizophora macronata*. However, the finding of this study was consistent with those of Komerek *et al.* (2007), who reported high level of Cr in roots than in shoot tissues. Earlier studies had pointed to the fact that hyper accumulator plants were not only tolerant to high concentrations of pollutants but also exhibited bioconcentration and translocation factors that are greater than one (Ma *et al.*, 2001). Finding of this study has shown that *R. racemose* dissipated a BTF < 1 , an observation that classify the plant as a non-hyper accumulator of Ni.

The study on Cd accumulation showed that *R. racemose* accumulated more concentrations of Cd in their shoot tissues than root tissues. The results were significant across stations at $p < 0.05$. The above finding is in tandem with an earlier observation by Pahalawattarachchi *et al.* (2011) who reported high concentrations of Cd, Zn, Ni and Pb in the shoots than roots of *Rhizophora macronata*. In their report, they stated low uptake capacity of the referenced metals studied. Similarly, Nirmal *et al.* (2011) reported high concentration of Cd in the leaves of *Avicennia marina*. The evaluated bio transfer and bio translocation factors of Cd in this study showed concentration > 1.0 . The findings in this study corroborate an earlier report by Ma *et al.* (2001) who observed that tolerant plants to high concentrations of pollutants equally exhibit bioconcentration and translocation factors > 1 . The finding of this study has indicated that *R. racemose* exhibited the characteristics of a hyper accumulator plant and consequently be classified as a hyper accumulator of Cd.

The study on Pb accumulation in the tissues of *R. racemosa* and showed that *R. racemosa* accumulated high levels of Pb in their shorts than root tissues. Although, the concentrations were not significantly different across stations. Various reports had indicated the accumulation of Pb in the shoot tissues of mangrove species (Pahalawattarachchi *et al.*, 2011; Nirmal *et al.*, 2011; Qiu *et al.*, 2011).

The bio transfer and bio translocation factors of Pb in *R. racemosa* shoot tissues were > 1 , which is an attribute of hyper accumulator plants. It is there inferred that *R. racemosa* is a hyper accumulator of Pb in shoot tissues.

The results on the evaluation of zinc (Zn) accumulation in the tissues that *R. racemosa* indicated an accumulation of substantial concentrations of Zn in root tissues, with a bio transfer factor > 1.0 . However, this result was not significantly different across stations at $p < 0.05$. Similarly, the bio translocation factors were not significant. Root tissues had been shown to accumulate higher concentration of most metals than shoot tissues (Pahalawattarachchi *et al.*, 2009; Nirmal Kumar *et al.*, 2013 and Almasheer *et al.*, 2014). The high levels of accumulation of metals in root tissues had earlier been attributed to low mobility and bioavailability of the metals to natural process of plant uptake (Komerek *et al.*, 2007). Consequent to the finding of this study, *R. racemosa* can be classified as a non-hyper accumulator of Zn in root tissues.

5 Conclusion

The phyto-extraction potential of selected metals by *R. racemosa* was evaluated in this study using bio transfer factor and bio translocation factor analysis. Findings of the study have shown that the study plant exhibited varying responses to the accumulation of the metals studied. The study unveiled that besides other attributes such as serving as a spooning habitat for various endemic fisheries and as well as home to many faunas in the Niger Delta mangrove forest, *R. racemosa* can be used for phytoremediation of the metals to which it shows high affinity and potentials for accumulation. The study indicated and classified *R. racemosa* as a hyper accumulator of Pb and Cd in their shoot tissues, and a non-hyper accumulator of Cr, Ni and Zn in their root tissues.

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