Journal of Applied Biosciences 94:8907 – 8914

ISSN 1997–5902

The potential of *Aspergillus fumigatus* and *Aspergillus niger* in bioaccumulation of heavy metals from the Chemu Lagoon, Ghana

Doku T. E. and Belford E. J. D*.
Department of Theoretical and Applied Biology, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
*Corresponding author: ejdbelford@yahoo.co.uk

Original submitted in on 17th September 2015. Published online at www.m.elewa.org on 31st October 2015
http://dx.doi.org/10.4314/jab.v94i1.12

ABSTRACT

Objectives: Bioaccumulation of heavy metals by fungi has been a major focus of most bioremediation studies owing to the excellent metal binding properties of the fungal cell wall. The capability of fungi isolated from sediments of the Chemu Lagoon to bioaccumulate heavy metals in lagoon water was investigated.
Methodology and Results: Fungi were isolated using pour plate technique and sub cultured to obtain pure cultures. Fungi isolated included *Aspergillus candidus, A. fumigatus, A. niger*, *A. tamarii*, *Mucor rouxii*, *Penicillium notatum* and *Rhizopus* sp. *Aspergillus fumigatus* and *Aspergillus niger* were selected for heavy metals bioaccumulation studies on PDB-amended with lagoon water in ratios of 1:1, 1:3 and 1:5 respectively for 3 weeks. Lead and Iron were the most bioaccumulated metals in *A. niger* and *A. fumigatus* with levels of 36.92 mg/L and 73.09 mg/L respectively. Bioaccumulation of heavy metals by both fungi during the wet season was higher than the dry and semi-wet seasons.
Conclusions and applications of findings: Both *Aspergillus fumigatus* and *Aspergillus niger* have good potential for the remediation of heavy metals in polluted water bodies. Comparatively *A. fumigatus* is a good candidate for Arsenic accumulation whilst *A. niger* is good candidate for Cadmium. The relatively longer period of inoculation of media with fungi is essential in increasing the levels of toxic metals accumulated by fungi. The ability of *A. fumigatus* and *A. niger* to bioaccumulate less toxic metals such as Iron, Lead and Zinc at high concentrations in the presence of more toxic metals could imply their versatility in tolerating and or bioaccumulating both classes of metals.
Key words: Bioaccumulation potential, fungi, heavy metals, *Aspergillus*, Chemu lagoon.

INTRODUCTION

The presence of heavy metals in aquatic ecosystems presents a serious environmental problem as they tend to accumulate in sediments from which macro-biota derive nutrition (Udofia *et al*., 2009). About twenty (20) metals including Lead, Arsenic, Cadmium and Zinc have been classified as toxic. These metals are among the first six metals that pose significant threat to human health as they form free radicals in living tissues, which cause degradation of DNA and lipid peroxidation (ATSDR, 2011). Microorganisms have been studied for their ability to remediate industrial wastewater containing heavy metals (Sag and Kutsal, 2001; Schröder, 2007; Ademola, 2009; Al-Garni *et al*., 2009; Iram *et al*., 2012). In sediments and soil mycelia fungi have the advantage of binding soil particles as their growth mode enables them to bridge soil pores (Harms *et al*., 2011). Moreover, fungi have wider surface area and excellent cell wall metal binding properties for the uptake of some heavy metals in solution (Bellion *et al*., 2006). Bioaccumulation of heavy metals in solution by living fungal cells offers better uptake of toxic metals like Cadmium and Arsenic than non-living biomass (Pal *et al*., 2010). *Aspergillus* species have been isolated from polluted sites and tested for their tolerance to high concentrations of toxic metals (Faryal *et al*., 2006; Javaid *et al*., 2011; Pandey and Banerjee, 2012; Shivakumar *et al*., 2011). Their potential in bioaccumulation of heavy metals is distinguished among fungi (Al-Garni *et al*., 2009). The Chemu Lagoon situated in Tema, an industrial hub in the Greater-Accra region of Ghana, serves as a sink for both untreated and semi-treated industrial and municipal effluents. The release of industrial effluents laden with heavy metals has compromised the quality of this aquatic ecosystem making it one of the most polluted water bodies in Ghana, resulting in a major loss of biodiversity. This research investigates the potential of *Aspergillus fumigatus* and *Aspergillus niger* to bioaccumulate heavy metals (As, Cd, Fe, Pb and Zn) in water from the Chemu Lagoon. The success of this approach will serve as the first step in the bioremediation process of the lagoon's restoration.

MATERIALS AND METHODS

Sampling site: The Chemu Lagoon is located in Tema, Ghana between latitudes 5°38'40.34'' N and 5°38'56.96'' N and longitudes 0° 01'16.61'' E and 0° 00'47.25'' E. It covers an area of about 26 km^2 with a number of tributaries joining the main stream. The catchment area has mangrove vegetation, salt flats, fresh water channels and salt-water channels. Sampling Site A was located upstream about 100 m from the open section of the lagoon, Site B (midstream), characterized by marshy area with mangrove vegetation. Sampling Site B is about 200 m from the mouth of the lagoon while Site C was located about 100 m downstream in the lagoon channel that joined the sea.

Sampling: Sampling was done at intervals of three months over a period of nine months in 2014, which corresponded with the rainfall patterns in Ghana. The first was carried out in April, during the dry season (DS), the second in July, during the wet season (WS) and the third in October, during the semi-wet season (SWS). Sediments were collected with the aid of sterile PVC pipes at a depth of 2 cm while water samples from the lagoon were obtained from the sub-surface using sterile bottles before midday and transported immediately to the SGS-Tema laboratory for heavy metal analysis according to APHA standards (2005).

Fungal isolates: A mixture of sediments and lagoon water was filtered (400-µm membrane) and 1 ml of the filtrate spread on the surface of Potato Dextrose Agar (PDA) plate and incubated at room temperature for 5 days. Fungal isolates were transferred to fresh PDA plates aseptically, using 7 mm diameter cork borer and incubated at room temperature for another 3 days. The fungal isolates were stained with lactophenol blue and identified at the Crop Science Department of the Faculty of Agriculture, KNUST.

Media amendment: Potato Dextrose Broth (PDB) was amended with filtrate of sediment and lagoon water in 100 ml flasks in ratios of 1:1, 1:3 and 1:5 to a volume of 50 mL. The flasks were inoculated with 7 mm diameter mycelia plugs of the respective fungi, shaken at intervals and incubated at 25 °C for 21 days.

Harvesting of mycelia and heavy metal analysis: Mycelia were harvested from flasks by filtration through 400-µm membrane and gently blotted with filter paper and dried at 40 ºC for 2 days. Nitric acid and chloric acid (HNO_3 and $HClO_3$) were used to digest 1 g of dried mycelia and made ready for heavy metal determination using Atomic Absorption Spectrophotometer (Varian Spectra model AA 55B).

Determination of bioaccumulation potential of fungi: Bioaccumulation potential of inoculated fungal isolates was calculated using the equation of Volesky and May-Phillips (1995) as follows:

Bioaccumulation potential

$$= [V\,(Cf - Ci)/Ci] \times M/1000$$

Where V= total volume of solution

Cf= final concentration of heavy metal in inoculated fungi

Ci= initial concentration of heavy metal in pure fungal culture

M= mass of fungus used for heavy metal digestion

RESULTS AND DISCUSSION

Fungal isolates : Fungi isolated from the lagoon included four *Aspergillus* species, namely *A. candidus*, *A. fumigatus*, *A. niger* and *A. tamarii*, as well as *Mucor rouxii*, *Penicillium notatum* and *Rhizopus sp*. *A. niger* and *A. fumigatus* were selected for studies of heavy metals bioaccumulation potential. These fungi have been isolated from polluted environments by other researchers and demonstrated ability to tolerate high metal concentration (Al-Garni *et al*., 2009; Ademola *et al*., 2009).

Bioaccumulation potential of selected fungi: The concentration of accumulated Fe, Zn, Pb, Cd and As by *A. niger* ranged from 0.19 to 30.87 mg/L, 0.03 to 27.86 mg/L, 0.04 to 36.92 mg/L, 0.04 to 18.14 mg/L and 0.04 to 12.28 mg/L respectively (Table 1). Lead (Pb) was the highest accumulated metal followed by Fe, Zn, As and Cd respectively in *A. niger*. On the other hand, the concentration of accumulated Fe, Zn, Pb, Cd and As by *A. fumigatus* ranged from 0.39 to 73.10 mg/L, 0.05 to 71.54 mg/L, 0.02 to 40.02 mg/L, 0.04 to 6.19 mg/L and 0.16 to 62.75 mg/L respectively (Table 1). Iron (Fe) was the highest accumulated metal followed by Zn, As, Pb and Cd respectively (Figure 3). The levels of bioaccumulated Iron were significantly higher in *A. fumigatus* compared to *A. niger*, especially in the wet season ($p < 0.05$) (Figure 3). The observed high bioaccumulation of Fe is directly related to its high concentrations in both water and sediment samples coupled with enzymatic functions in cells (Deacon, 2006). Thus, it is more bioavailable to a large extent although most fungi secrete siderophores to absorb iron (Fe^{3+}) in their environment (Schrettl *et al*., 2008). The levels of Zinc, Arsenic and Lead bioaccumulated in *A. fumigatus* were significantly higher compared to *A. niger* especially during the semi-wet season ($p < 0.05$) (Figures 1, 2 and 4). Zn is a micronutrient adsorbed in higher concentrations compared to toxic metals like Arsenic and Cadmium but excess concentrations initiate pathway to block its uptake into the cell (Gitan *et al*., 2003; Shivakumar, 2012). Although Lead, Arsenic and Cadmium have no obvious metabolic relevance in fungi, they are taken up through both intracellular and extracellular chelating reactions by wall components (Bellion *et al*., 2006; Das *et al*., 2007). It was observed that Lead accumulation increased as amendment concentrations increased (Figure 2). Similar finding was recorded by Shivakumar *et al*. (2012) and Chen and Wang (2007). Higher media amendments (1:3 and 1:5) recorded higher levels of accumulation of lead by both fungi (Figure 2). More so, its bioaccumulation varied significantly on the seasonal basis implying that lead could have strong associations in dry and moist sediment conditions. Unlike Pb and Cd, which tend to destabilize the structure of fungal membrane molecules by forming strong complexes, Arsenic was not accumulated to appreciable levels. Arsenic bioaccumulation increased as amendment concentrations increased although it has deleterious effects on fungal growth. This observation has no definite mechanism supporting its occurrence although exudation of organic acids tends to lower the ambient pH thereby to increasing bioavailability and uptake of some toxic metals (Ademola, 2009). More so, in as much as these toxic metals destabilize membrane structure, they induce stress, which generates many responses but one key aspect of this reaction is the secretion of mucilaginous binding molecules that have high affinity for these metals (Pócsi, 2011). The levels of bioaccumulated cadmium were significantly higher in *A. niger* compared to *A. fumigatus* ($p < 0.05$) (Figure 5).

Table 1: Mean concentration of heavy metal accumulated by *Aspergillus fumigatus* and *Aspergillus niger*

		Concentration of heavy metal (mg/L)					
Metal	Treatment	Dry season		Wet season		Semi-wet season	
		A. fumigatus	*A. niger*	*A. fumigatus*	*A. niger*	*A. fumigatus*	*A. niger*
Fe	**Control**	**0.012 ± 0.001^{a}**	**0.024 ± 0.070^{b}**	**0.065 ± 0.003^{a}**	**0.012 ± 0.002^{a}**	**0.060 ± 0.007^{a}**	**0.002 ± 0.0005^{a}**
	1:1	0.626 ± 0.006^{f}	0.553 ± 0.099^{f}	73.098 ± 11.102^{i}	28.453 ± 2.118^{h}	0.395 ± 0.016^{b}	10.379 ± 0.191^{k}
	1:3	0.720 ± 0.070^{f}	0.237 ± 0.066^{e}	33.973 ± 5.169^{f}	30.874 ± 1.88^{hi}	30.702 ± 2.237^{f}	4.878 ± 0.034^{g}
	1:5	0.448 ± 0.021^{e}	0.195 ± 0.026^{d}	28.364 ± 5.211^{e}	30.628 ± 2.447^{hi}	18.154 ± 1.729^{e}	6.508 ± 0.271^{i}
Zn	**Control**	**0.012 ± 0.002^{a}**	**0.004 ± 0.057^{a}**	**0.012 ± 0.002^{a}**	**0.014 ± 0.003^{a}**	**0.011 ± 0.004^{a}**	**0.014 ± 0.003^{a}**
	1:1	0.166 ± 0.031b^{c}	0.218 ± 0.0216^{e}	33.864 ± 9.100^{f}	20.743 ± 1.345^{f}	1.754 ± 0.076^{b}	1.711 ± 0.051^{d}
	1:3	0.273 ± 0.057^{d}	0.091 ± 0.01^{cd}	26.823 ± 5.350^{e}	27.862 ± 2.60^{g}	71.537 ± 6.245^{j}	0.910 ± 0.002^{cd}
	1:5	0.048 ± 0.009^{a}	0.034 ± 0.005^{b}	14.797 ± 6.265^{c}	24.267 ± 2.128^{g}	11.491 ± 1.459^{d}	1.211 ± 0.121^{c}
Pb	**Control**	**0.013± 0.004^{a}**	**0.004 ± 0.004^{a}**	**0.014 ± 0.008^{a}**	**0.004 ± 0.001^{a}**	**0.014 ± 0.003^{a}**	**0.004 ± 0.0006^{a}**
	1:1	0.017 ± 0.006^{a}	0.163 ± 0.007^{d}	0.237 ± 0.024^{b}	8.436 ± 1.525^{d}	1.165 ± 0.050^{b}	0.444 ± 0.014^{b}
	1:3	0.082 ± 0.007^{ab}	0.037 ± 0.004^{b}	24.412 ± 6.013^{d}	33.549 ± 1.525^{i}	16.295 ± 1.897^{e}	0.696 ± 0.029^{bc}
	1:5	0.195 ± 0.013^{cd}	0.057 ± 0.005^{c}	25.059 ± 2.045^{d}	36.926 ± 3.944^{j}	40.015 ± 9.949^{g}	0.925 ± 0.127^{cd}
Cd	**Control**	**0.004 ± 0.002^{a}**	**0.003 ± 0.001^{a}**	**0.006 ± 0.001^{a}**	**0.003 ± 0.001^{a}**	**0.006 ± 0.001^{a}**	**0.001 ± 0.0009^{a}**
	1:1	0.041 ± 0.009^{a}	0.048 ± 0.009^{b}	0.727 ± 0.108^{b}	1.820 ± 0.266^{b}	6.134 ± 0.152^{c}	7.906 ± 0.126^{j}
	1:3	0.045 ± 0.010^{a}	0.036 ± 0.005^{b}	0.596 ± 0.095^{b}	18.136 ± 1.581^{f}	5.328 ± 0.574^{c}	4.438 ± 0.050^{f}
	1:5	0.048 ± 0.011^{a}	0.040 ± 0.002^{b}	0.224 ± 0.009^{a}	6.688 ± 0.271^{cd}	6.186 ± 0.556^{c}	5.921 ± 0.156^{h}
As	**Control**	**0.001± 0.001^{a}**	**0.002 ± 0.0001^{a}**	**0.001 ± 0.001^{a}**	**0.002 ± 0.0002^{a}**	**0.001 ± 0.0005^{a}**	**0.002 ± 0.0007^{a}**
	1:1	0.160 ± 0.046^{ab}	0.223 ± 0.078^{e}	1.746 ± 0.247^{b}	0.051 ± 0.020^{a}	1.754 ± 0.105^{b}	0.717 ± 0.012^{bc}
	1:3	0.206 ± 0.070^{cd}	0.041 ± 0.011^{b}	3.675 ± 1.247^{g}	12.275 ± 1.778^{e}	53.675 ± 5.476^{h}	1.735 ± 0.250^{d}
	1:5	0.243 ± 0.077^{cd}	0.046 ± 0.007^{d}	2.752 ± 0.162^{h}	3.609 ± 0.822^{b}	62.752 ± 9.620^{i}	2.317 ± 0.231^{e}

Mean±SD in the same column having different letters differ significantly ($p < 0.05$)

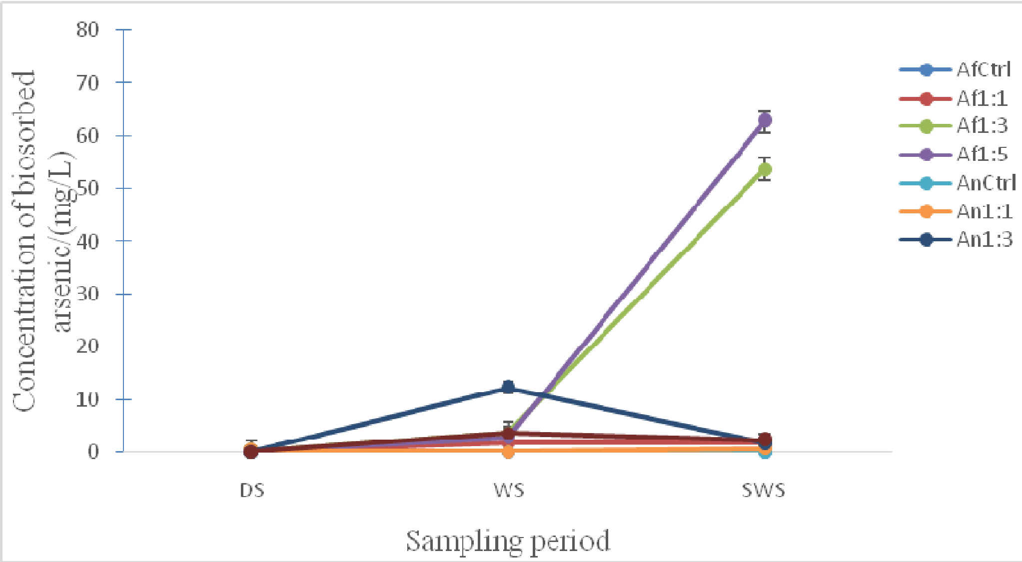

Figure 1: Seasonal variation of Arsenic accumulated by *A. fumigatus* and *A. niger*

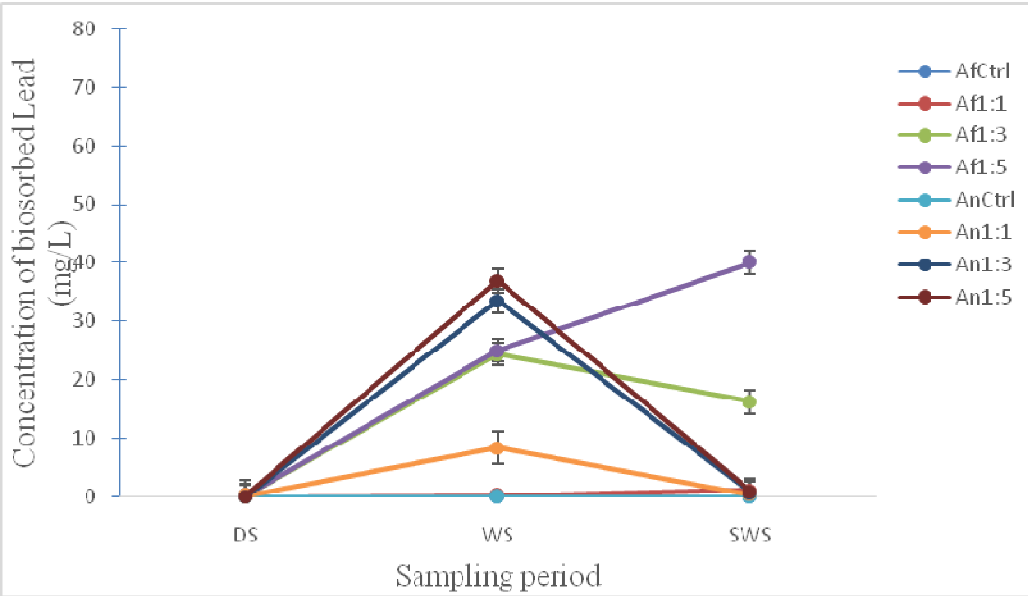

Figure 2: Seasonal variation of Lead accumulated by *A. fumigatus* and *A. niger*

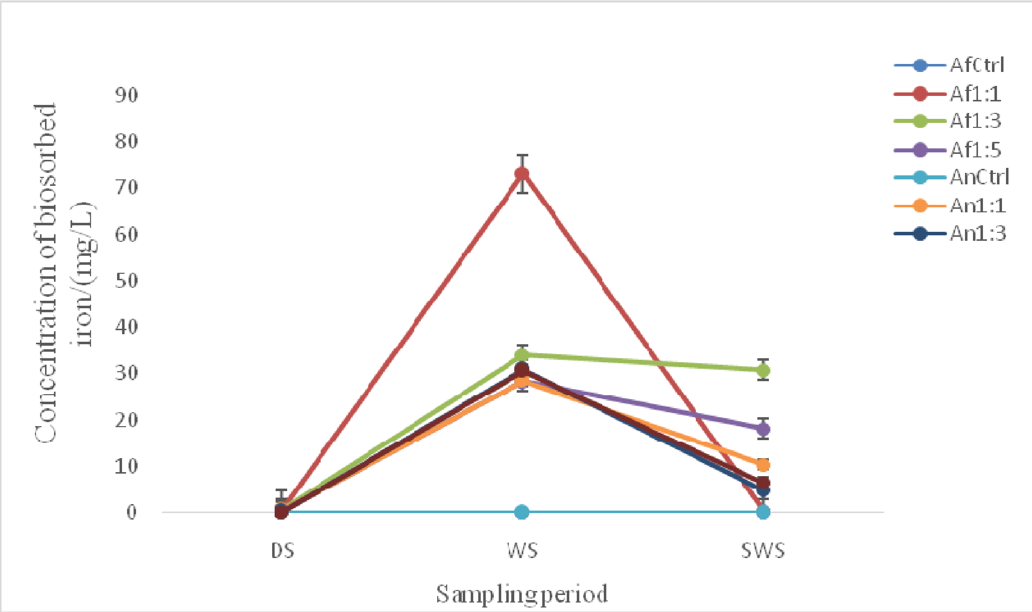

Figure 3: Seasonal variation of Iron accumulated by *A. fumigatus* and *A. niger*

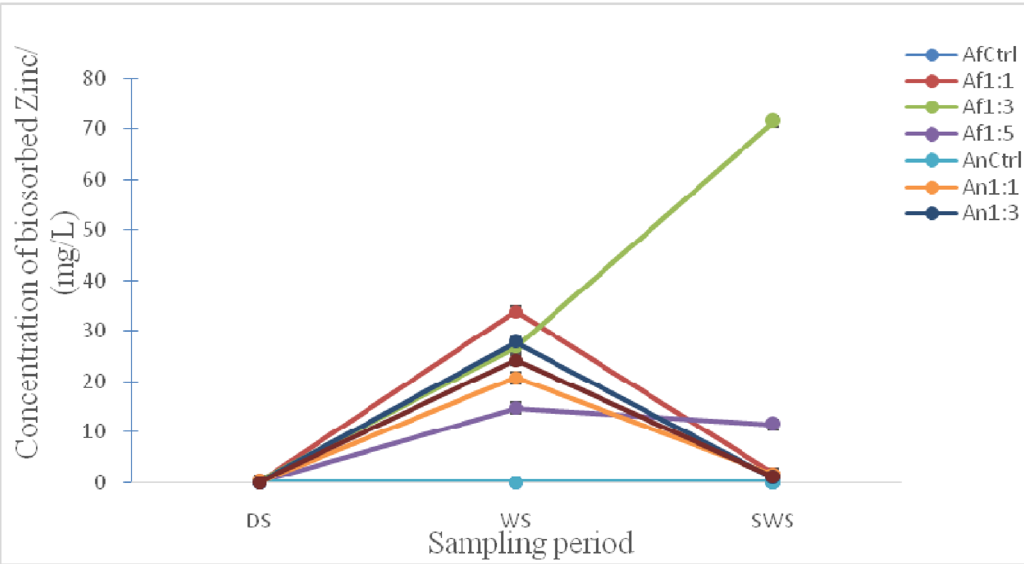

Figure 4: Seasonal variation of Zinc accumulated by *A. fumigatus* and *A. niger*

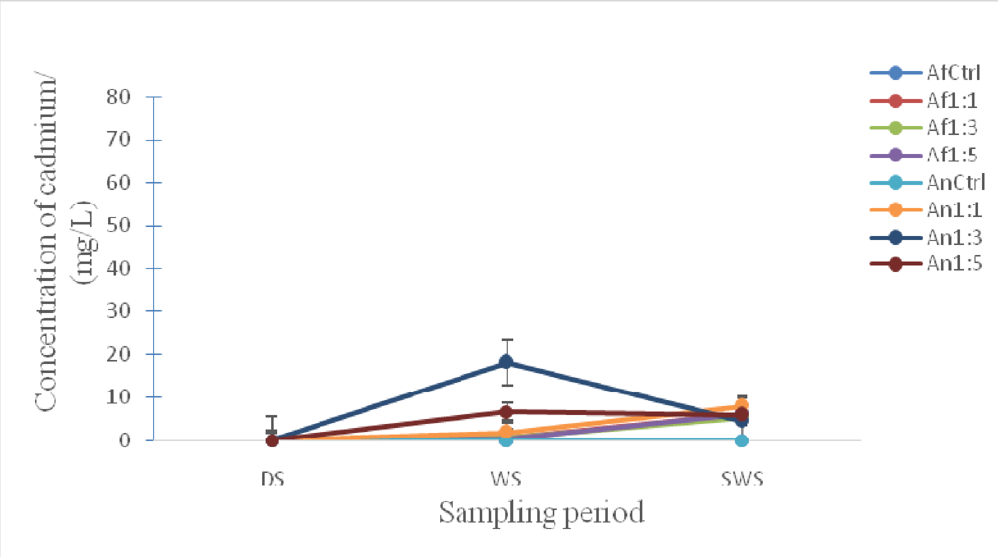

Figure 5: Seasonal variation of Cadmium accumulated by *A. fumigatus* and *A. niger*

Although the level observed is appreciable compared to earlier reports oxidative stress induction by cadmium is known to elicit metal chelation or exclusion leading to its low uptake from substrates (Chakraborty *et al*., 2014; Al-Garni *et al*., 2009). Cadmium bioaccumulation could also have been hampered because extracellular precipitation, complexation and binding reduce bioavailability of Cadmium (Lanfranco *et al*., 2004; Pócsi, 2011) although fungal growth was observed with no significant bioaccumulation taking place. Quite distinguishing about the potential of bioaccumulation by these fungi is their ability to actively take up relatively none toxic metals such as Iron, Zinc and Lead with the toxic metals such as Cadmium and Arsenic also being adsorbed to appreciable levels. Seasonal effect of sampling has also been observed in this study to affect the bioavailability of metals and their subsequent bioaccumulation. This is tentative in that temperature variation affects the occurrence of metals in water bodies especially as it tends to affect other key physicochemical parameters (Del Ramo *et al*., 1987). The second sampling (WS) which was conducted in the wet season had the highest bioaccumulation potential for both fungi and this could imply that rainfall impacts directly, or indirectly on some key physiochemical parameters affecting the bioavailability of metal ions in polluted environment.

CONCLUSION

Nature provides answers to resolve environmental pollution and this study affirms the potential of *A. fumigatus* and *A. niger* in the bioaccumulation of heavy metals from the Chemu lagoon. Bioaccumulation (active uptake of materials by living biomass) is very promising in terms of removing relatively non-toxic metals such as Iron, Lead and Zinc but is hampered by deleterious effects of toxic metals. *Aspergillus fumigatus* exhibited better bioaccumulation potential in the uptake of both relatively non-toxic and toxic metals showing marked uptake concerning Arsenic. *A. niger* also demonstrated good level of bioaccumulating less toxic metals but with marked accumulation of Cadmium. The combination of both fungi in the bioremediation process will ensure a more efficient clean up of heavy metals in the lagoon. However, accumulation of excessive levels of these metals could be cytotoxic to the fungi and other living organisms. Both fungi could be further studied to identify physiological factors that improve the bioaccumulation of metals.

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