Journal of Applied & Environmental Microbiology, 2018, Vol. 6, No. 2, 30-36
Available online at http://pubs.sciepub.com/jaem/6/2/1

DOI:10.12691/jaem-6-2-1

Arbuscular Mycorrhizal Fungi Diversity in Selected Heavy metal Contaminated Soils in Owerri, Nigeria

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Abstract An investigation was carried out on the species diversity of Arbuscular mycorrhizal (AM) fungi in selected heavy metal contaminated soils within Owerri metropolis. Soils (0-20cm) were collected from 8 different locations viz (1) high auto mechanic activity area. (2) Medium auto mechanic activity area. (3) Low auto mechanic activity area. (4) Old dump site. (5) Active dump site. (6) Recovery dump site. (7) Heavy traffic highway. (8) Undisturbed vegetative land that served as control. Soil samples were analyzed for physicochemical parameters including heavy metal viz lead (Pb), Cadmium (Cd) and Zinc (Zn). Also the ecological characteristics of AM fungi species were determined. Results showed that areas with high pollution index (PI) adversely affected the richness and diversity of AM fungi species. Seven different AM fungi ecotypes were isolated from the soil samples. These ecotypes had varied relative abundance across the locations. Species richness and diversity as measured by Shannon-Wiener index decreased in soils with PI > 20 but increased with low PI < 20. However, these organisms maintained significant populations across the locations. Thus suggesting high possibility of facilitating microbial activity in heavy metal stressed soil ecosystems.

Keywords: *Abuscular Mycorrhizal fungi (AMF), species diversity, land degradation, soil restoration and heavy metals*

Cite This Article: Chris O. Nwoko, Chinwe J. Ngumah, Chinedu E. Ihejirika, Chima Ngumah, and Adaeze A Ojiaku, "Arbuscular Mycorrhizal Fungi Diversity in Selected Heavy metal Contaminated Soils in Owerri, Nigeria." *Journal of Applied & Environmental Microbiology*, vol. 6, no. 2 (2018): 30-36. doi: 10.12691/jaem-6-2-1.

1. Introduction

Arbuscular mycorrhizal fungi of phylum Glomeromycota are obligate biotrophic organisms that establish symbiotic relationships with the roots of angiosperms, gymnosperms and some bryophyte and fern species [1]. They are important components in soil ecosystem, as these fungi species can contribute to plant growth by reducing stresses resulting from nutrients deficiencies mainly phosphorous (P), pollution and drought [2].

They have been considered critical components for the efficient recovery of degraded lands, primarily through inoculation of sites with effective isolates [3]. The major function of mycorrhizae is nutrient transport. Extra-radical hyphae anchored in the roots exploit soil outside the root where it absorbs mineral nutrients (mainly N, P, and micronutrients) translocate them back to the root and transfer them to the host plant in exchange for photosynthetically fixed carbon in the form of sugar. Abuscular mycorrhizal fungi can effectively detoxify both organic and inorganic pollutants. However, the efficiency of this method of remediation, depends on the species and origin of the fungi used, the type of plant colonized and the concentration of the contaminant [4]. Arbuscular mycorrhizal (AM) fungi is an extra-ordinarily important soil microbes since they increase nutrient acquisition by the plant as well as helping plants overcome biotic and abiotic stress [5,6]. Arbuscular mycorrhizal fungi also help in the improvement of water relations, soil stability and the limitation of heavy metal uptake by plants.

Soil may become contaminated by accumulation of heavy metal and metalloids through emissions from the rapidly expanding industrial activity, disposal of high metal wastes and vehicular emissions, burning of fossil fuels, mining and smelting of metallifrous ores, municipal wastes, fertilizers, pesticides, sewage sludge amendments, the use of pigments and batteries. Some of these metals are micronutrients necessary for plant growth such as Zn, Cu, Mn, Ni and Co. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be immobilized. There are also reports of AM fungi from metal contaminated soil which suggests potential adaptation of the indigenous AM population. Several heavy metal tolerant, arbuscular mycorrhizal fungi have been isolated from polluted soils, which can be useful for reclamation of such degraded soils as they are found to be associated with a large number of plant species in heavy metal polluted soil etc. The presence of toxic metals in soils can severally inhibit the biodegradation of organic contaminants. Heavy metal contaminated soil

may pose risks and hazards to human and the ecosystem through direct ingestion [5]. Soil degradation causes a shrinking of arable land resources and may lead to persistence starvation and malnutrition [7]. The preservation of good soil quality is a key factor for sustainability of ecosystem. This applies also to non-agricultural ecosystem where enhancing soil quality can help to restore disturbed sites, reduce the risk of erosion and store carbon in the soil [8]. Two important aspects of soil quality are the soil structure and the soil fertility which are influenced by the presence of arbuscular mycorrhizal (AM) fungi. Soil structure is therefore of pivotal importance for ecosystem functioning and can contribute to ecological land restoration, erosion prevention and carbon stabilization in the soil [8,9]. Gildon and Tinker [10] isolated a mycorrhizal strain which tolerated 100 g kg^{-1} of Zn in the soil. Similarly, Weissenhorn *et al*, [11] isolated mycorrhizal fungi from two heavy metal polluted soil which were found to be more resistant to Cd than a reference strain. Also AM fungal species has been reported by Griffioen *et al*, [12] from the rhizosphere of *Agwstis capillaries* growing in contaminated surroundings of Zn refinery in the Netherlands. This indicated that these fungal have evolved Zn and Cd tolerance and that they might play an important role in conferring Zn and Cd tolerance in plants. Mycorrhizal fungi have also been shown to be associated with metallophyte plants on highly polluted soils where only adapted plants such as *Viola calaminiaris* (violet) can grow. Also *Glomus sp* isolated from the roots of the violet plant improved maize growth in a polluted soil and reduced root and shoot heavy metal concentrations. It has been suggested that heavy metal tolerant, AM fungi could protect plants against harmful effects of excessive heavy metals. Several biological and physical mechanisms have been proposed to explain metal tolerance of AM fungi and AM fungal contribution to heavy metal tolerance of host plants. Immobilization of metals in the fungal biomass is one such mechanism involved. Arbuscular mycorrhizal fungi metal tolerance includes adsorption onto plant or fungi cell walls present on and in plant tissues, or into extra radical mycelium in soil, chelation by such compounds as siderophores and metallothionens released by fungi or other rhizosphere microbes and sequestration by plant derived compounds like phytochelation or phytates [13]. Other possible metal tolerance mechanism include dilution by increased root or shoot growth, exclusion by precipitation onto polyphosphate granules into particles or other membrane rich organelles, indirect mechanism include the effect of AM fungi on rhizosphere characteristics such as changes in pH, microbial communities and root exudation patterns. The aim of this research work was to determine the diversity and other ecological characteristics of arbuscular mycorrhizal fungi species in selected heavy metal contaminated soils in Owerri metropolis.

2. Materials and Methods

2.1. Climate of the Study area

The study was carried out within Owerri metropolis, the capital city of Imo state. It lies within the Nigeria humid rain forest belt, which ecologically is more prominent for tuber than cereal production. It is situated between latitudes $5^{o}29^{1}$ N, longitudes $7^{o}02^{1}$E and elevation 300 meters.

Owerri city is underlain by the Benin Formation as shown in the geologic map of Imo River Basin (Figure 1). This formation consists of coastal plain sands, which is about 0.05 – 2.0 mm in size, with minor clay beds. It contains some isolated gravels, conglomerates, and very coarse sandstone in some places. Thickness of the formation is about 800m at its depocenter, while the mean depth to water table is about 24m [14]. Benin Formation is overlain by alluvium deposits and underlain by Ogwashi-Asaba Formation which consists of lignite, sandstones, clays and shale.

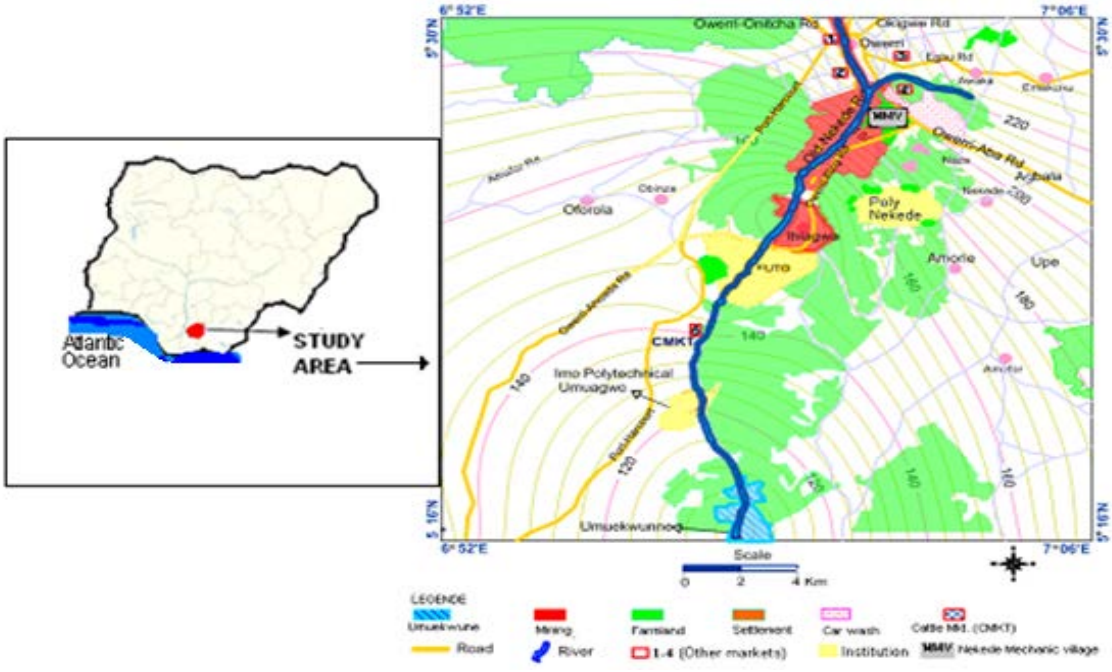

Figure 1. Map of Nigeria showing Imo state and Owerri Municipal

2.2. Soil Sample Collection

Samples were collected from selected ecologically degraded soils in Owerri. The following eight sites were selected for evaluation viz: (a) Soil from high Auto-mechanic activity (b) soil from medium Auto mechanic activity (c) soil from low auto-mechanic activity, (d) soil from old dump site (e) soil from active dumpsite (f) soil from recovering dumpsite, (g) soil from traffic highway (h) soil from undisturbed vegetated land (served as control).

From the contaminated areas, rhizospheric soils (0-20cm) were randomly selected at 3 different points per location and bulked to obtain composite soil. Sampling was carried out on May, 15, 2016. A total of 8 soil samples were collected from all the locations. The soil samples were brought to the laboratory in sterile condition and stored in a refrigerator at 4°C until analysis.

2.3. Isolation and Identification of Mycorrhizal Spores.

Each sampling point was analyzed separately, to determine total number of spores in 100 g of dry soil. Sub-soil samples were removed to assess soil physical and chemical characteristics including selected heavy metals (Pb, Cd, Zn).

The number of AM Fungi spores was determined by wet sieving [15], The wet screening technique was conducted by weighing 50 g of soil samples and then dissolved in 50 ml of water and left for 10-15 minutes to allow the sediment to settle. The suspension was then filtered by pouring it into sieves of pores 250µm, 200 µm and 150µm in sequence. The supernatant was carefully and slowly emptied into a flask and from there 10 ml aliquot was taken to a petri dish and observed for presence of spores in a stereoscope (40x). The spores were then counted and quantified.

For the identification, a slide of the supernatant was prepared and was observed in a microscope (40x). Each spore type was mounted sequentially in water, lactophenol, PVA (polyvinyl lactic acid) and Melzer's reagent [16]. Identification was based on spore color, size, surface ornamentation and wall structure and identification was made to the species level, with the aid of the [17] manual, and descriptions provided by the site of International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http:// invam.caf.wvu.edu) and the original species descriptions.

2.4. Quantification of Arbuscular Mycorrhizal Spores

1. The spores were quantified by direct counting. The supernatant suspension was transferred into a test tube and made up to 50 ml, vortexed, and out of which 1ml was transferred into a watch glass. This step was performed three more times to count spores in four replicates. Situations where the number of spores in watch glass was much to be counted comfortably, dilutions were increased and recounted.
2. Average counts were obtained from four watch glasses and results were multiplied by volume of aliquot (x total mls in test tube) and expressed as number of spores / 50 g soil.

2.5. Soil Heavy Metal and Chemical Properties Determination

2.5.1. Sample Digestion

Five gram of each soil sample was digested with a mixture of 10mls of concentrated HCl and 10mls of concentrated HNO_3. The mixture was digested until a clear digest was obtained. During the digestion, the heating was progressively increased. On completion of the digestion, the digest was diluted with deionized water, filtered and made up to 50mls with deionized water in standard polythene graduated flask. The solutions were then used to determine the concentration of the required heavy metals using Atomic Absorption Spectrophotometer –Thermo Scientific model**.** Total nitrogen was determined by the Kjeldahl digestion and distillation procedure as described in Soil Laboratory Staff Manual (1984). Available Phosphorus was determined using [18] methods as modified by [19]

2.6. Ecological and Statistical Data analysis

The ecological characteristics of the Arbuscular mycorrhizal species were estimated as follows: species richness (R), evaluated by the number of arbuscular mycorrhizal fungal species (NS) present in 50 g of dried soil, Simpson's dominance index (ʅ), and Shannon-Wiener diversity (H'), evenness, relative abundance (Table 1). The NS, R, and H' data were also subjected to analysis of variance (ANOVA), using the Minitab version 16 statistical software and significant means were compared using Turkey's least significant different test ($p<0.05$). Pearson coefficient of correlation (r) was used to compare soil chemical properties on AM diversity. The Pollution index was determined using the equation by [20].

$$PI= HC_1 \times HC_2 \times HC_3 \ldots\ldots\ldots\ldots\ldots\ldots (HC_n)^{1/n}$$

Where PI = heavy metal pollution index, HC is the concentration of *n* heavy metals in soil samples

Table 1. Ecological characteristics of AM Fungi and techniques for calculation

s/n	Parameters	Formula
1	Abundance (ni)	Number of spores per species
2	Relative abundance (pi)	RA = Number of spores for the species (genus) x 100% / Total number of spores identified
3	Species richness	Number of species per samples
4	Simpson's dominance index (ʅ)	$ʅ = \Sigma ni(ni-1) / N(N-1)$
5	Shannon –Wiener (H')	$H' = -\Sigma Pi \log_2 Pi$ *
6	Species Evenness (J')	$J' = H' / \log_2 S$*

*Pi = n_i/N, where n_i = number of spores of species, *i;* N = total number of spores identified, H' max = $\log_2$ **S,** where **S =** total number of species (genera) identified.

3. Results and Discussion

3.1. The AM Fungi Species Isolated and Their Relative Abundance in the Study Area

The result revealed the presence of *Glomus mossae* in almost all the locations (Table 2). Other species such as *Glomus claroideum, Gigaspora sp, Scutellospora sp* and *Acaulospora sp* were also present in most of the locations. The undisturbed forest (H) had greater diversity of the AM fungi present when compared to the different locations .The *Glomus mossae* was more in abundance in almost all the locations.

3.2. The Ecological Characteristics of AM fungi in the Study Area

All the study locations had varied number of AM species in the soils. The undisturbed forested area had the highest species richness (7.16±0.2) Table 3. The least richness was recorded in the recovering dumpsite (3.03±0.05). The same trend was observed in the species diversity, the recovering dumpsite had the least diverse AM species (0.54±0.00) while the undisturbed forested area had the highest diversity of 2.76 ± 0.00 (Table 3). Also location H with highest richness and diversity had relative abundance of *Glomus mossae* (0.12), *Glomus claroideum* (0.2), *Glomus sp1*(0.1), *Glomus sp2*(0.1), while location F recorded *Glomus claroideum* (0.38), *G.sp 1*(0.34), *Gigaspora sp* (0.28). Simpson species dominance did not follow similar trend with Shannon-wiener index (Table 3). AM species were much more dominant (0.51) in location D -soil of old dumpsite and showed very low dominance in the undisturbed forested area (0.13). Simpson species dominance significantly differed in all sampling locations with old dumpsite having the highest dominance. The species evenness significantly differed ($P<0.05$) in all the locations. The medium automechanic activity showed an even (0.92 ±0.00) AM species distribution in all the study locations. This is evident in the species relative abundance of *Gigaspora sp* (0.4), *Scutellospora sp(0.28), G.mossae (0.52)*. All the study locations had species evenness in the following order B(0.92) > A(0.91) > D(0.53) > C(0.48) > E(0.41). From the results the following locations had species diversity >1 and followed the trend H > G > B > A > D > C > E > F.

Table 2. Arbuscular mycorrhizal fungi species identified from the selected locations

s/n	Location	Geo-reference	AM fungi identified and their relative abundance
1	A	Lat N 5 27'' 35'' Long E 7 2'' 7''	*Glomus mossae (0.5), Glomus claroideum(0.17), Gigaspora sp(0.33)*
2	B	*Lat N 5 27'' 31''* *Long E 7 2'' 7''*	*Glomus mossae(0.28), Scutellospora sp(0.4), G.versiforme (0.12), Acaulospora sp(0.2)*
3	C	*Lat N 5 27'' 48''* *Long E 7 2'' 10''*	*Glomus claroideum(0.29), G. sp 1(0.12), G.mossae(0.41) , Acaulospora sp(0.18),*
4	D	*Lat N 5 28'' 5''* *Long E 7 2'' 4''*	*Gigaspora sp(0.4), Scutellospora sp(0.28), G.mossae(0.52)*
5	E	*Lat N 5 28'' 5''* *Long E 7 2'' 26''*	*Glomus mossae(0.19), G.sp 1(0.28), G.sp 2(0.14), Gigaspora sp(0.38)*
6	F	*Lat N 5 28'' 6''* *Long E 7 2'' 30''*	*Glomus claroideum(0.38), G.sp 1(0.34), Gigaspora sp(0.28)*
7	G	*Lat N 5 26'' 57''* *Long E 6 59'' 22''*	*Glomus mossae(0.19), Glomus claroideum(0.22), G.sp1(0.08), G sp 2(0.08), Gigapora sp(0.19), Scutellospora sp(0.22),*
8	H	*Lat N 5 26'' 57''* *Long E 6 59'' 24''*	*Glomus mossae(0.12), Glomus claroideum(0.2), Glomus sp1(0.1), Glomus sp2(0.1), Scutellospora sp(0.2), Acaulospora sp(0.15)*

A= Soil from Low Auto-mechanic activity, B=Medium auto-mechanic activity, C=high Auto mechanic activity area, D=soil from old dumpsite, E=active dumpsite, F=recovery dumpsite, G= heavy traffic highway, H= undisturbed forested area (control).

Table 3. Species richness, species diversity of Arbuscular mycorrhizal fungi in the study area

Location	Species richness	Species diversity	Evenness	Simpson
A	3.2 ± 0.36^{e}	1.44 ± 0.01^{c}	0.91 ± 0.00^{c}	0.36 ± 0.00^{b}
B	3.9 ± 0.11^{d}	1.85 ± 0.01^{b}	0.92 ± 0.00^{b}	0.26 ± 0.00^{d}
C	4.0 ± 0.05^{d}	0.96 ± 0.00^{e}	0.48 ± 0.00^{f}	0.25 ± 0.00^{e}
D	4.0 ± 0.00^{d}	1.06 ± 0.00^{d}	0.53 ± 0.00^{e}	0.51 ± 0.00^{a}
E	4.93 ± 0.11^{c}	0.82 ± 0.00^{f}	0.41 ± 0.00^{g}	0.24 ± 0.00^{f}
F	3.03 ± 0.05^{e}	0.54 ± 0.00^{g}	0.34 ± 0.00^{h}	0.31 ± 0.00^{c}
G	6.16 ± 0.2^{b}	1.86 ± 0.00^{b}	0.72 ± 0.00^{d}	0.16 ± 0.00^{g}
H	7.16 ± 0.2^{a}	2.76 ± 0.00^{a}	0.98 ± 0.00^{a}	0.13 ± 0.00^{h}

A= Soil from Low Auto-mechanic activity, B=Medium auto-mechanic activity, C=high Auto mechanic activity area, D=soil from old dumpsite, E=active dumpsite, F=recovery dumpsite, G= heavy traffic highway, H= undisturbed forested area (control).
Column having the same letters did not significantly differ at 5% probability

Table 4. Concentration of heavy metal (Cd, Zn, Pb) in the study area

Location	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	PI
A	3.33 ±0.00^{b}	23.45± 0.05^{c}	176.36± 0.05^{c}	23.96
B	2.41 ±0.00^{d}	21.49 ±0.01^{f}	155.72 ±0.02^{d}	20.05
C	2.06 ± 0.03^{f}	59.58 ±0.03^{a}	242.44± 0.05^{a}	30.98
D	3.31± 0.00^{b}	19.28 ±0.03^{g}	157.6± 0.00^{c}	21.58
E	2.37± 0.00^{e}	22.49± 0.02^{e}	91.37 ±0.00^{e}	16.95
F	1.31± 0.00^{g}	23.29 ±0.03^{d}	187.44± 0.05^{b}	17.88
G	3.38 ±0.10^{a}	53.08± 0.02^{b}	51.65 ±0.05^{f}	21.00
H	3.016±0.00 ^{c}	7.91 ±0.00^{h}	52.53± 0.05^{f}	10.78

A= Soil from Low Auto-mechanic activity, B=Medium auto-mechanic activity, C=high Auto mechanic activity area, D=soil from old dumpsite, E=active dumpsite, F=recovery dumpsite, G= heavy traffic highway, H= undisturbed forested area (control) . PI= pollution index
Column having the same letters did not significantly differ at 5% probability.

3.3. The Soil Chemical Characteristics and Heavy Metal Concentration of the Study Locations

The concentration of the different heavy metals (metalloids) in the soil varied among the locations (Table 4). Soil from location F had the highest concentration of Zn (187.44mg/kg) followed by location A (176.36mg/kg). Location F had the lowest concentration of Cd (1.31mg/kg) while location G had the highest concentration of Pb (53.08mg/kg). The heavy metal pollution index ranged from 10.78 to 30.98. The highest HM contamination occurred in location C (High auto-mechanic activity area) followed by location A (low auto-mechanic activity) while the lowest pollution index was recorded in the control, undisturbed forested area (H). Heavy metal pollution index at the high auto- mechanic activity recorded highest value (30.98) followed by low auto-mechanic activity (23.9) and the least value of 10.78 in the undisturbed forested area (Table 4).

The cation exchange capacity of the soil varies according to the clay percentage, the type of clay, soil pH and amount of organic matter. Soils with high CEC typically have high organic matter content which are considered to be more fertile, as they can hold more plant nutrients; which could be attributed to the fact that organic matter colloids have qualities of negative charges. From the results CEC ranged from 496.8± 0.01Cmol/kg to 2288.5 ± 0.17Cmol/kg (Table 5). All the soils from the study locations recorded CEC above recommended minimum of 120Cmol/kg.

Soil organic carbon (SOC) is the main source of energy for soil microorganisms. The ease and speed with which SOC becomes available is related to the soil organic matter (SOM) fraction in which it resides. Location F (recovery dumpsite) had the highest org. C of 2.46±0.05% and the lowest (1.03±0.02%), from the medium auto-mechanic activity. Percentage total N was low in the undisturbed soil compared to all other disturbed soil.

3.4. Relationship between the Physicochemical Parameters and the Ecological Parameters of AM Fungi

There was significant ($p<0.001$) negative correlation between total phosphorus and species richness and diversity of AM (Table 6). Similar trend was recorded in organic carbon, total nitrogen and Zn against evenness. However, Cd and pH had significant positive relation with species richness, diversity and evenness (Table 6). Also Electrical Conductivity (EC), and the Cation Exchange Capacity (CEC) and Pb (mg/kg) did not influence any of the ecological characteristics.

Table 5. Soil chemical characteristics of the study locations

Location	CEC (Cmol/kg)	P (mg/kg)	Org. C(%)	N (%)	pH	EC (µS/cm)
A	1980.5 ±0.17^{c}	25.9±0.08^{b}	1.96± 0.05^{b}	0.131 ±0.01^{c}	5.59± 0.05^{c}	0.28± 0.05^{d}
B	615.5± 0.12^{e}	24.3±0.02^{c}	1.03 ±0.02^{f}	0.124± 0.05^{e}	5.79 ±0.05^{b}	0.21± 0.05^{g}
C	644.6±0.07^{d}	20.4±0.11^{e}	1.38± 0.02^{d}	0.134± 0.05^{b}	5.49 ±0.01^{e}	0.32± 0.03^{b}
D	548.7±0.06^{g}	22.2±0.11^{d}	1.47 ±0.05^{c}	0.126± 0.05^{d}	4.48± 0.01^{h}	0.42± 0.03^{a}
E	569.3±0.06^{f}	16.7±0.07^{f}	1.07 ±0.01^{f}	0.120± 0.05^{f}	4.91± 0.05^{g}	0.22 ±0.02^{f}
F	2288.5±0.17^{a}	31.2±0.04^{a}	2.46± 0.05^{a}	0.150 ±0.05^{a}	5.19± 0.01^{f}	0.20± 0.03^{h}
G	496.8±0.01^{h}	6.51±0.01^{h}	0.86 ±0.06^{g}	0.037 ±0.05^{h}	5.5 ±0.05^{d}	0.24 ±0.04^{e}
H	2154.2±0.06^{b}	7.71±0.05^{g}	1.17± 0.07^{e}	0.042± 0.05^{g}	6.66± 0.01^{a}	0.29 ±0.04^{c}

A= Soil from Low Auto-mechanic activity, B=Medium auto-mechanic activity, C=high Auto mechanic activity area, D=soil from old dumpsite, E=active dumpsite, F=recovery dumpsite, G= heavy traffic highway, H= undisturbed forested area (control) . PI= pollution index
Column having the same letters did not significantly differ at 5% probability.

Table 6. Correlation coefficient of several chemical parameters and some ecological characteristics of AM spores.

s/n	Variable	Species richness	Species diversity	Evenness	Simpson diversity
1	P (total)	-0.961***	-0.672***	NS	NS
2	Org. C	-0.687***	-.0.535**	NS	NS
3	N (total)	-0.932***	-0.802***	-0.519**	NS
4	pH (H_20)	0.544**	0.828***	0.737***	-0.538**
5	CEC	NS	NS	NS	NS
6	EC (µS/cm)	NS	NS	NS	NS
7	Cd (mg/kg)	0.439*	0.568**	0.611**	NS
8	Pb (mg/kg)	NS	NS	NS	NS
9	Zn (mg/kg)	-0.631***	-0.645***	-0.432*	NS

Ns=not significant, ***=0.1%, *=5%, ** =1%.

3.5. Discussion

The auto-mechanic activities and waste management strategies affected the soil heavy metal load which significantly accounted for the low species richness and diversity of Arbuscular mycorrhizal (AM) fungi populations in some locations. The total AM richness decreased in locations that recorded high pollution index compared to that of low pollution index but the propagules and spores never disappeared completely from these areas indicating some level of adaptation of these indigenous AM fungi to such environmental stress. It is noteworthy that AM population correlated well with metal ions especially Cd^{2+} and Zn^{2+}, except Pb^{2+}, these metal ions are absorbed by soil microbes and perceived to be toxic at moderate to high concentrations. Griffioen, *et al.*, [13] in their experiment on AM fungi and Cu observed no correlation between the concentration of these metals in sludge-amended agricultural soils and AM fungi populations.

Despite their availability, methods to measure free-ion activity in the soil solution have rarely been used in studies relating to heavy metal and AM fungi. The use of such methods as a reference for comparison would probably help elucidate the reason for the discrepancies found between different studies. Arbuscular mycorrhizal fungi species richness also correlated negatively with the P and organic carbon content of the soil (Table 6) , a result that is well documented in the literature [21,22].

Species richness and Shannon-wiener diversity index did not show any definite trend in relation to the different locations, but one fact stands out, locations with high PI recorded low richness and diversity. This level of AM propagule diversity observed in contaminated locations could be a fungal stress response whereby fungal ecotypes better adapted to unpolluted soil but affected at intermediates rates of contamination allow other fungi, probably less competitive in non-stressed soils but better adapted to heavy metals, to colonize the roots of plants and complete their life cycles. Several heavy metal-tolerant AM fungi have been isolated from polluted soils, which can be useful for reclamation of such degraded soils as they are found to be associated with a large number of plant species in heavy metal-polluted soil. Gildon and Tinker [23] isolated a mycorrhizal strain which tolerated 100 mg kg^{-1} of Zn in the soil. Considerable amount of AM fungal colonization was also reported in an extremely polluted metal mining area with HCl-extractable Cd soil concentration of more than 300 $mgkg^{-1}$ [11].

Cd-tolerant *G. mosseae* from metallophyte plant enhanced growth of maize, alfalfa, barley, etc. in heavy metal-rich soils [24]. Thus, mycorrhizal fungi adapted to elevated soil metal concentration can significantly improve the growth and plant P nutrition under metal stress. By maintaining a higher shoot P/Zn concentration ratio mycorrhizal plants are able to alleviate the negative effects of Zn [25]. Therefore, isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems [4,26].

It has been suggested that heavy metal-tolerant AM fungi could protect plants against harmful effects of excessive heavy metals [27]. Several biological and physical mechanisms have been proposed to explain metal tolerance of AM fungi and AM fungal contribution to metal tolerance of host plants. (a) Immobilization of metals in the fungal biomass is one of such mechanisms involved [28]. (b)Reduced transfer, as indicated by enhanced root/ shoot Cd ratios in AM plants [29]. This may occur due to intracellular precipitation of metallic cations with PO_4.

The Pearson's correlation analysis shows that the increase in soil organic carbon significantly and negatively correlated ($P < 0.05$) with species richness and diversity of AM fungi community. pH also significantly correlated with species richness and diversity. We observed highest AM richness and diversity in sites with high soil pH especially the control. Although, the occurrence of Acaulosporaceae is observed in soils with a low pH [30], we assume that member of this genus may be present in soils with a wide pH range, as reported by [31] who observed *Acaulospora* species in soils where the pH ranged from 4.9 to 6.4.

Soils usually contain more than one species of AM fungi [6]. In a single rhizosphere, it is possible to find six to eight different morphotypes [32]. The species were not unique to any study location. Through Simpson's index of dominance (> 20%), we confirmed the presence of all AM fungi species in all the locations.

Cardozo Júnior *et al.*, [33] noted that a community with high diversity is characterized by low dominance. This was true for H (undisturbed vegetation, which exhibited a greater Shannon –wiener diversity (2.76) and a lower Simpson's dominance (ℶ = 0.13). Wu *et al.*, [34] also

noted that plant species correlated positively with the abundance of AM fungi. It is possible that the community structure of the AM species in locations with high PI was affected by the dominance of species in the AM fungi communities combined with the dynamics of ecological succession related to plants in the development stage.

From this study we recorded lowest richness values in sites with a higher concentration of phosphorus in the soil (Table 3). On the other hand, the high P level and other heavy metals values determined maybe helpful in the selection of AM fungi species adapted to these conditions. This finding has relevance with respect to facilitating isolation studies and the use of these species in the introduction of seedlings of native or exotic plants inoculated with AM fungi, both for environmental restoration or agricultural activities.

4. Conclusion

Auto-mechanic activities and poor waste management strategies affected heavy metal load of the urban soils which accounted for the low species population and diversity of AM fungi ecotypes. These AM fungi ecotypes seem to tolerate heavy metal stressed environment as evidenced in the significant presence of AM propagules in all the locations. This observation could be helpful in the phytoremediation of metals/metalloids laden soils.

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