SHST
Horticulture and Forestry Society of Transylvania

Osaigbovo M *et al.* (2022)
Notulae Scientia Biologicae
Volume 14, Issue 2, Article number 11236
DOI:10.15835/nsb14211236
Research Article

NSB
Notulae Scientia Biologicae

Influence of two soil fungal inocula on growth of *Abelmoschus esculentus* (L.) Moench. plants grown in arsenic-treated soils

Marquis OSAIGBOVO[1]*, Theophilus A. DANIA[2], Emuejevoke D. VWIOKO[2]

[1]*Government Science and Technical College, Irrua, Edo State, Nigeria; marquimbik100@gmail.com (*corresponding author)*
[2]*University of Benin, Faculty of Life Sciences, Department of Plant Biology and Biotechnology, Benin City, Edo State, Nigeria; theophilus.dania@uniben.edu; vwioko@yahoo.com*

Abstract

In this study, growth of *Abelmoschus esculentus* (L.) Moench. was investigated in soils treated with arsenic ions and inoculated with two fungi two weeks after planting. *Aspergillus niger* and *Aspergillus terreus* were selected out of five isolated fungi from a heavy metal contaminated soil and used to inoculate soils treated with, 50,100 and 150 ppm As^{3+} ion solutions. *Abelmoschus esculentus* plant growth was measured, where data were collected for germination, morphological and reproductive characters, chlorophyll content, activities of nitrate reductase, catalase (CAT), and super oxide dismutase (SOD). Soil enzymes i.e., urease, phosphatase, dehydrogenase and cellulases were monitored. Results showed that the two fungal inocula increased number of leaves. Plant height was increased in both 100 and 150 ppm As^{3+} treated soils from 19.50 cm to 26.86 cm and 17.60 cm to 30.97 cm in uninoculated and *Aspergillus terreus* inoculated soils, respectively. Delayed flowering was observed in plants grown in the As^{3+} treated soils. The fungal inocula affected SOD and CAT activities in leaves of *Abelmoschus esculentus*, in As^{3+} treatments. At 50ppm and 100ppm, inoculation with *A. niger* and *A. terreus* significantly reduced SOD activity. Fungal inoculation stimulated phosphatase and cellulases production in the As^{3+} treated soils. This study clearly shows that the soil inoculants immensely contributed to alleviating heavy metal stress encountered by plants.

Keywords: arsenic; *Aspergillus*; fungi; heavy metals; okra; super oxide dismutase

Introduction

Soil is one of the most important resources for human survival and development. It is also one of the most endangered parts of our environment, that is exposed to a variety of environmental pollutants arising from human, industrial and agricultural activities (Morton-Bermea *et al.*, 2002). Materials and chemicals from mine tailings, high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater from irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition can accumulate and cause soil contamination and pollution (Khan *et al.*, 2008, Zhang *et al.*, 2010).

Received: 01 Apr 2022. Received in revised form: 26 May 2022. Accepted: 31 May 2022. Published online: 14 Jun 2022.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Heavy metals constitute a group of soil pollutants, which are dangerous to plant and animal health. Heavy metal contamination of the environment is a global problem; because they are able to accumulate in the different parts of food chain and cannot be easily broken down as organic pollutants (Doumett *et al.*, 2008). Heavy metals found at contaminated sites include lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) in order of abundance (USEPA, 1996).

Arsenic is non-essential element and it is generally considered toxic to plants. The roots are usually the first plant part to be exposed to arsenic, where it inhibits root extension and proliferation. Upon translocation to the shoot, arsenic can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production (Garg and Singla, 2011; Vwioko and Edobor, 2017). Arsenic availability in soil can disturb normal functioning of plant metabolism, consequently leading to stunted growth and low crop productivity. At high concentrations, arsenic interferes with important metabolic processes, which can lead to plant death (Nel *et al.*, 2006; Shahid *et al.*, 2015).

Arsenic is also reported to inhibit the rate of photosynthesis in plants (Nagajyoti *et al.*, 2010; Gusman *et al.*, 2013). Cellular membranes in plants become damaged when exposed to arsenic, causing electrolyte leakage (Singh *et al.*, 2006). The exposure of plants to arsenic induces the production of reactive oxygen species (ROS), like superoxide ($O_2\bullet-$), the hydroxyl radical ($OH^{\bullet}$), and H_2O_2 (Ahsan *et al.*, 2008; Mallick *et al.*, 2011). Reactive oxygen species can damage proteins, amino acids, purine nucleotides and nucleic acids and cause peroxidation of membrane lipids (Moller *et al.*, 2007). Several researchers (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1995; Abedin *et al.*, 2002; Liu *et al.*, 2005) have reported some symptoms of arsenic toxicity in plants to include, inhibition of seed germination, reduction in plant height and root length, wilting and necrosis of leaf blades, decrease in shoot growth and lower fruit and grain yield. Chandra *et al.* (2016) reported percent reductions in yield of the okra plants subjected to various As(III), As(V) and DMA treatments.

Fungi were reported by some researchers for their role in improving plant tolerance in heavy metal contaminated soils. Ascomycota and Basidiomycota are the most commonly reported fungi groups to detoxify heavy metals and improve the quality of heavy metal contaminated soils (Narendrula-Kotha and Nkongolo, 2017). However, it has been also observed that nutrient poor but heavy metal contaminated soils are often primarily colonized by arbuscular mycorrhizal (AM) fungi (Khan *et al.*, 2000). Fungal associations have been reported by various authors to improve the growth of different plants under various heavy metal (Cd/Ni) stresses. The plants include: Arabidopsis (Ike *et al.*, 2007), Rapes (Deng *et al.*, 2011), *Solanum nigrum* (Xiao *et al.*, 2010), *Festuca arundinacea* and *Festuca pratensis* (Soleimani *et al.*, 2010), *Lolium arundinaceum* (Ren *et al.*, 2011).

Salehi *et al.* (2016) reported that mycorrhizal inoculation improved survival, growth and volume production of white poplar plants grown on Pb-polluted soils. Kanwal *et al.* (2016) also reported that inoculation with mycorrhizal fungal species in plants had positive impacts on increasing the growth, biomass, yield, and nutrient contents of plants. They observed better growth performance, biomass, improved nutrients uptake and higher yields in mycorrhizal treated plants as compared to non-mycorrhizal treated wheat plants grown on zinc polluted soils. Jiang *et al.* (2016) stated that both *Glomus versiforme* and *Rhizophagus intraradices* inoculation greatly improved plant growth by increasing phosphorus (P) acquisition and improved activities of antioxidant enzymes in AMF-inoculated *Lonicera japonica* planted in Cd-contaminated soil. Khan and Lee (2013) have reported that under Cu stress, inoculated soybean plants, had significantly higher Chlorophyll a, b and total carotenoid as compared to non-inoculated soybean plants. Zhang *et al.* (2002) and Massaccesi *et al.* (2002) have suggested that strains/species of *Penicillium* can mitigate Cd and other metal-related toxicity, which is attributed to their potential to produce bioactive metabolites or enzymes. Massaccesi *et al.* (2002) noted that *Penicillium funiculosum* can produce gibberellins which can contribute to the ability of a fungus to convert the toxic metal into stable complexes.

Okra (*Abelmoschus esculentus* (L.) Moench) belongs to the family *Malvaceae* and genus *Abelmoschus*. It is known in many English-speaking countries as lady's fingers. The okra plant can be cultivated in tropical, subtropical and warm temperate regions of the world and, can be grown throughout the year (Abidi *et al.*, 2014). Okra can be grown on wide range of soils, but well drained fertile soils with adequate organic matter produces high yield (Akinyele and Temikotan, 2007). The chromosome number of okra, ($2n$ = 72, 108, 120, 132 and 144) are in regular series of polyploids with n = 12 (Abidi *et al.*, 2014). Okra is grown for its pods, which are rich in fibre and vitamins (Andras *et al.*, 2005). The objective of this study was to assess the performance of *Abelmoschus esculentus* plants grown in arsenic-treated soil inoculated with *Aspergillus niger* and *Aspergillus terreus*.

Materials and Methods

Plant material

The seeds of *Abelmoschus esculentus* L. (Moench.) were collected from a local farm at Uselu, Benin City, Edo State, Nigeria.

Soil sampling for fungi isolation

Soil samples for isolation of fungi were collected from a car spray painter shop, along Ewah Road, New Benin, Benin City, Edo State, Nigeria.

Isolation of fungi

Fungi were isolated from the collected soil samples according to Waksman (1927) on PDA (Potato Dextrose Agar) plates. The PDA plates were incubated at room temperature (32 °C) for 2 to 3 days. Then, colonies of fungi on the PDA plates were isolated and sub-cultured. Pure cultures of five different isolates were obtained using fresh PDA plates. Pure cultures of these isolates were sub-cultured and kept on slants for further use.

Identification of fungi

The fungal isolates were morphologically identified and characterized by observing their morphological characteristics using lactophenol stain, spores and hyphae as well as the presence or absence of septa etc., and coloured monographs were recorded and compared with those that appeared in St-Germain and Summerbell (1996), Ellis and Ellis (1996) and Barnett and Barry, (1998).

Growth of fungi species in As^{3+} supplemented media

The fungi isolates were inoculated on media supplemented with different concentration of arsenic III trioxide, As_2O_3. The concentrations applied were: 0 ppm (control), 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. Daily mycelia extension (cm) of the isolates in culture plates was measured for three days. All treatments were replicated three times.

Preparation of As^{3+} ion solutions

The arsenic salt used was provided in the form of arsenic III trioxide. The different weights of As_2O_3 were dissolved in deionized water to prepare specific concentrations in parts per million (ppm). For example, 200 mg, 400 mg and 600 mg of As_2O_3 were dissolved in 4 litres of deionized water to give 50 ppm, 100 ppm and 150 ppm respectively.

Greenhouse experiment

A total of thirty-six (36) plastic bowls were used in the experiment. Soil was air-dried and sieved to remove stones and other particles. The soil was collected from the Faculty of Agriculture demonstration farm, University of Benin. Three kilogrammes (3 kg) of the sieved soil were then weighed and packaged in each perforated plastic bowl.

Application of As^{3+} solutions to experimental pots

Each experimental pot was first watered with deionized water up to the water holding capacity, and left to drain excess water overnight and later with 200ml of prepared solutions of As^{3+} and left for 24 hours before planting. Planting was done at a rate of 10 seeds per pot.

Soil inoculation with fungi

Two fungi isolate with higher mycelia extension were selected, from the results of the screening growth on heavy metal supplemented media and were used for inoculation in the greenhouse experiment, two weeks after planting. Fungi broth culture suspensions were prepared by inoculating PDA broth with the fungus and incubating the cultures for 3 to 5 days at 32 °C. After incubation period, cultures were examined using the hemocytometer analysis to determine the number of spores. The spore suspension was standardized to 16×10^5 spores/ml by adding 10ml of the culture to 90ml of sterile distilled water. The standardized spore suspensions were used to inoculate the soils in pots.

Experimental design

Pots were arranged in a completely randomized using triplicate pots in each treatment.

Germination

The appearance of plumule above the soil surface was recorded as germination. The number of seeds that germinated per pot were counted and percent germination was calculated. The germination record was taken every day after sowing for 12 days. The height of plants (21-days old) in each pot was measured. Measurements were taken every two weeks. The number of leaves formed on a plant were counted and recorded every two weeks. The number of buds formed on a plant were counted and the *number of days to flowering* was recorded when the first flower was observed. The number of flowers formed on a plant were recorded. The number of fruits formed on a plant in were counted and recorded.

Physiological plant parameters

In order to measure the total chlorophyll content, 0.25 g of leaf tissues were ground in cold mortar in darkness and adjusted to volume 25 ml by 80% acetone and centrifuged at 3000 rpm for 10 mins. The supernatant was spectrophotometrically examined (using Biobase, UV-VIS spectrophotometer BK-UV1800pc), at 645 nm and 663 nm wavelengths. To estimate total chlorophyll and chlorophyll a, b by spectrophotometry, the following equations were used (Arnon, 1949; Gross, 1991).

The chlorophyll content was calculated with the following formula:

$$chlorophyll\ a(mg/g\ tissue) = \frac{(11.75A_{662} - 2.350A_{645})\ \text{x V}}{1000\ \text{x W}}$$

$$chlorophyll\ b(mg/g\ tissue) = \frac{(18.61A_{662} - 3.960A_{645})\ \text{x V}}{1000\ \text{x W}}$$

V= final volume

W= weight of tissue used

Total chlorophyll content = Chlorophyll a (mg/g tissue) + Chlorophyll b (mg/g tissue)

Nitrate reductase analysis (NR) in leaf was done according to the procedure of Hageman and Huckles by (1971) with slight modifications. A weight of 0.5 g fresh root was crushed in a test tube containing 3.0 ml of 0.05M potassium phosphate buffer (pH-7.8) and 3.0ml of 0.4 M KNO_3 solution and kept in the dark. At the end of incubation period (2 hours), tubes were kept in boiling water bath for 5 min to stop the enzyme activity and complete leaching of the nitrite in the medium. Nitrite was estimated by the method of Evans and Nason (1953) where the absorbance was read at 540nm, using spectrophotometer (Biobase, UV-VIS spectrophotometer BK-UV1800pc). The standard calibration curve was prepared using sodium nitrite solution.

Catalase (CAT) activity was assayed according to the methods of Korolyuk *et al.* (1988). The reaction was started by the addition of 0.1mL of tissue sample to 1ml of 4% ammonium molybdate and 2 ml of 0.03% H_2O_2 solution. One unit of catalase activity is defined as the amount of enzyme required to clear 1µmol of H_2O_2 per minute per gram of tissue. The breakdown of hydrogen peroxide in the reaction mixture was measured spectrophotometrically at 410 nm.

Superoxide dismutase was measured adopting the methods described by Misra and Fridovich (1972).

Soil phosphatase activity was determined following the procedure described by Eivazi and Tabatabai (1977).

Urease activity in the soils was determined by the standard procedures outlined by Broadbent *et al.* (1958). Dehydrogenase activity was assayed according to the methods outlined by Casida *et al.* (1964).

Cellulase activity was estimated according to the methods described by Pancholy and Rice (1973).

Statistical analysis

Mean and standard deviation were calculated from the data collected. Two-way ANOVA was carried out for the data using SPSS (version 16). Significant differences in mean values were indicated using Duncan Multiple Range (DMR) (Alika, 2006).

Results

Table 1 shows the morphological characteristics used to identify the fungi isolates. According to their characteristics these isolates could be identified as strains of Aspergillus *fumigatus, A. terreus, A. niger, A. flavus* and *Penicillium chrysogenum.* The growth of the isolates on different arsenic supplemented PDA media is shown in Table 2 as the mean colony diameter (cm). The mean colony diameters of the isolates had the following pattern: *A. fumigatus*>*A. niger*>*A. terreus*>*A. flavus*>*P. chrysogenum.* The widest colony diameter was recorded with *A. fumigatus*, while the narrowest was that recorded with *P. chrysogenum.* Three days after inoculation, the mean colony diameters in 100 ppm As-PDA media for *A. fumigatus, A. terreus, A. niger, A. flavus* and *Penicillium chrysogenum* were 5.57 cm, 4.57cm, 4.50 cm, 4.17 cm and 1.67 cm respectively.

Table 1. Morphological characteristics of the fungi isolates

Fungi isolate	Morphological characteristics	Microscopic features	Fungi identified
A	Coffee black mycelia, with mycelia concentrically arranged.	Smooth conidia	*Aspergillus fumigatus*
B	Light grey mycelia	Smooth conidia	*Aspergillus terreus*
C	Black mycelia, with white edges	Smooth conidia	*Aspergillus niger*
E	Brown mycelia	Globose conidia, rough conidia	*Aspergillus flavus*
G	Dark grey mycelia	Sub-globose conidia	*Penicillium chrysogenum*

Table 2. The mean colony diameter of the fungal isolates on PDA media supplemented with different concentrations of As^{3+} solutions

Treatments	Organisms	Mean diameter growth in Petri dish (cm)		
		1st DAI	2nd DAI	3rd DAI
0 ppm	A	4.20 ± 0.00	6.60 ± 0.00	8.20 ± 0.00
	B	4.23 ± 0.98	5.67 ± 0.12	6.00 ± 0.00
	C	3.67 ± 0.64	5.50 ± 0.75	6.47 ± 0.12
	E	2.27 ± 0.31	3.80 ± 0.60	5.57 ± 0.98
	G	1.60 ± 0.35	1.77 ± 0.32	1.90 ± 0.26
20 ppm As	A	2.63 ± 0.42	6.97 ± 0.25	7.93 ± 0.51
	B	2.70 ± 0.00	3.60 ± 0.00	5.10 ± 0.00
	C	4.50 ± 1.73	5.97 ± 0.67	7.67 ± 1.44
	E	2.50 ± 0.26	3.53 ± 0.21	4.07 ± 0.46
	G	1.73 ± 0.59	2.40 ± 0.53	2.63 ± 0.49
40 ppm As	A	1.97 ± 0.31	6.53 ± 0.29	7.67 ± 0.58
	B	2.43 ± 0.21	4.37 ± 1.40	5.33 ± 0.92
	C	3.57 ± 0.98	5.83 ± 1.26	6.70 ± 1.10
	E	3.30 ± 0.96	4.77 ± 1.15	5.43 ± 0.95
	G	1.63 ± 0.31	2.03 ± 0.29	2.17 ± 0.32
60 ppm As	A	2.73 ± 0.12	5.83 ± 1.47	7.70 ± 0.85
	B	2.30 ± 0.30	4.33 ± 0.72	5.43 ± 0.06
	C	2.97 ± 0.35	4.67 ± 0.42	6.00 ± 0.53
	E	2.67 ± 0.23	4.37 ± 0.75	4.60 ± 0.69
	G	1.83 ± 0.57	2.53 ± 0.76	2.63 ± 0.76
80 ppm As	A	2.10 ± 0.30	5.20 ± 1.06	5.80 ± 1.25
	B	2.37 ± 0.21	4.63 ± 0.42	5.10 ± 0.35
	C	3.73 ± 1.47	5.50 ± 0.70	6.13 ± 0.61
	E	2.77 ± 0.25	4.23 ± 0.35	4.77 ± 0.55
	G	1.80 ± 0.36	2.07 ± 0.32	2.23 ± 0.29
100 ppm As	A	2.10 ± 0.30	4.63 ± 0.75	5.57 ± 0.25
	B	2.20 ± 0.36	3.90 ± 0.17	4.57 ± 0.12
	C	2.07 ± 0.38	3.77 ± 0.70	4.50 ± 0.70
	E	2.13 ± 0.31	3.57 ± 0.97	4.17 ± 0.68
	G	1.20 ± 0.17	1.50 ± 0.20	1.67 ± 0.25

DAI: Days After Inoculation, values are mean ± S.D. A: *Aspergillus fumigatus* **B:** *Aspergillus terreus* **C:** *Aspergillus niger* **D:** *Aspergillus flavus* **E:** *Penicillium chrysogenum*

Table 3 shows the mean percent germination of *Abelmoschus esculentus* seeds in soils treated with different concentrations of As^{3+} ion solutions. Mean percent germination was observed to be below 50%, generally with the highest germination percentage recorded in control soils. The seeds obtained from the farmer showed weak germination ability. The number of leaves formed per plant is shown in Table 4. The number of leaves decreased progressively as the concentrations of As^{3+} solution applied to the soil increased without inoculation.

Table 5 shows the heights of Okra plants grown in soils with different levels of As contamination. Higher concentrations of As^{3+} applied to soils affected plant growth and reduced the plant height. Improved plant height was recorded in the soil inoculated with fungi. Notably at 150 ppm, mean plant height of non-inoculated soils was 17.60 cm, while height of plants grown in soil inoculated with *A. niger* was 28.50 cm, and for those grown in soil inoculated with *A. terreus* was 30.97 cm. Stem girth decreased with increasing concentrations of As^{3+}. Inoculation with fungi, increased stem girth in plants grown in As^{3+} supplemented soils.

The highest number of buds produced was observed in plants grown in 100ppm As^{3+} soils inoculated with *A. terreus*. At higher concentrations of As^{3+}, most of the plants failed to form flowers and fruits.

Table 3. Percent germination of *Abelmochus esculentus* seeds sown in soils treated with As^{3+} ion solutions

Treatment	Conc.	Days After Planting (DAP)				
		4	6	8	10	12
Control	-	15.56 ±18.10	37.77 ± 21.67	37.77 ± 21.67	37.77 ± 21.67	37.77 ± 21.67
As	50 ppm	4.44 ± 5.27	22.22 ± 15.63	22.22 ± 15.63	22.22 ± 15.63	22.22 ± 15.63
As	100 ppm	13.33 ± 12.25	27.78 ± 13.94	27.78 ± 13.94	27.78 ± 13.94	27.78 ± 13.94
As	150 ppm	14.44 ± 11.30	31.11 ± 7.82	31.11 ± 7.82	31.11 ± 7.82	31.11 ± 7.82

Values are mean ± S.D

Table 4. Number of leaves of *Abelmoschus esculentus* plants grown in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*

Treatments	Soil inoculants	Weeks After Planting (WAP)				
		3	6	9	11	13
0 ppm	None	3.00 ± 0.00	4.67 ± 1.15	7.00 ± 3.46	10.33 ± 5.77	13.33 ± 5.86
	A. niger	3.00 ± 0.00	4.00 ± 0.00	6.00 ± 1.00	10.00 ± 1.00	15.00 ± 3.00
	A. terreus	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	8.00 ± 1.00	10.00 ± 1.00
50 ppm As	None	3.00 ± 0.00	4.33 ± 0.58	5.33 ± 0.58	6.33 ± 0.58	9.00 ± 2.00
	A. niger	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	6.00 ± 0.00	10.00 ± 0.00
	A. terreus	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	8.67 ± 2.89	11.00 ± 3.46
100 ppm As	None	2.00 ± 0.00	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	8.00 ± 0.00
	A. niger	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	8.00 ± 0.00	11.00 ± 0.00
	A. terreus	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00
150 ppm As	None	4.00 ± 0.00	5.00 ± 0.00	6.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.00
	A. niger	4.00 ± 0.00	5.00 ± 0.00	6.00 ± 0.00	10.00 ± 0.00	14.00 ± 0.00
	A. terreus	3.00 ± 0.00	4.00 ± 0.00	5.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00

Values are mean ± S.D.

Table 5. Plant height of *Abelmoschus esculentus* plants grown in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*

Treatments	Soil inoculants	Weeks After Planting (WAP) (cm)				
		3	6	9	11	13
0 ppm	None	10.20 ± 0.00	13.40 ± 0.00	17.10 ± 0.00	19.50 ± 0.00	22.30 ± 0.00^{ef}
	A. niger	13.47 ± 5.04	19.13 ± 4.46	22.87 ± 2.41	24.47 ± 2.70	25.20 ± 2.05^{cde}
	A. terreus	10.63 ± 1.78	16.23 ± 3.60	19.87 ± 1.55	20.77 ± 1.53	23.30 ± 1.11^{e}
50 ppm As	None	9.10 ± 0.00	11.30 ± 0.00	18.50 ± 0.00	21.50 ± 0.00	23.90 ± 0.00^{de}
	A. niger	7.70 ± 0.00	8.50 ± 0.00	10.10 ± 0.00	12.50 ± 0.00	15.00 ± 0.00^{h}
	A. terreus	9.30 ± 0.00	17.40 ± 0.00	18.90 ± 3.35	24.63 ± 3.35	27.57 ± 5.01^{bc}
100 ppm As	None	8.30 ± 0.00	10.50 ± 0.00	14.90 ± 0.00	18.40 ± 0.00	19.50 ± 0.00^{fg}
	A. niger	8.10 ± 0.00	10.20 ± 0.00	12.10 ± 0.00	16.10 ± 0.00	24.20 ± 0.00^{de}
	A. terreus	5.60 ± 0.00	8.50 ± 0.00	15.50 ± 0.00	21.57 ± 0.64	26.87 ± 1.15^{bcd}
150 ppm As	None	5.50 ± 0.00	7.90 ± 0.00	12.10 ± 0.00	13.00 ± 0.00	17.60 ± 0.00^{gh}
	A. niger	10.00 ± 0.00	12.20 ± 0.00	14.50 ± 0.00	18.50 ± 0.00	28.50 ± 0.00^{ab}
	A. terreus	5.60 ± 0.00	8.50 ± 0.00	19.07 ± 0.98	28.87 ± 1.15	30.97 ± 1.85^{a}

Values are mean ± S.D.

Total chlorophyll content of leaves is shown in Figure 1. Without As^{3+} ion treatment applied to soil; the inoculated organisms improved chlorophyll content of plants by over 30% when compared to the control. Growing plants in As^{3+} treated soils decreased the mean values of chlorophyll content. Whereas plants grown in the presence of *Aspergillus terreus* in 50 ppm As^{3+}, 100 ppm $As^{3+}$and 150 ppm As^{3+}treated soil showed improvement in total chlorophyll content reaching 16.2%, 32.7% and 21.5% increase against the control respectively.

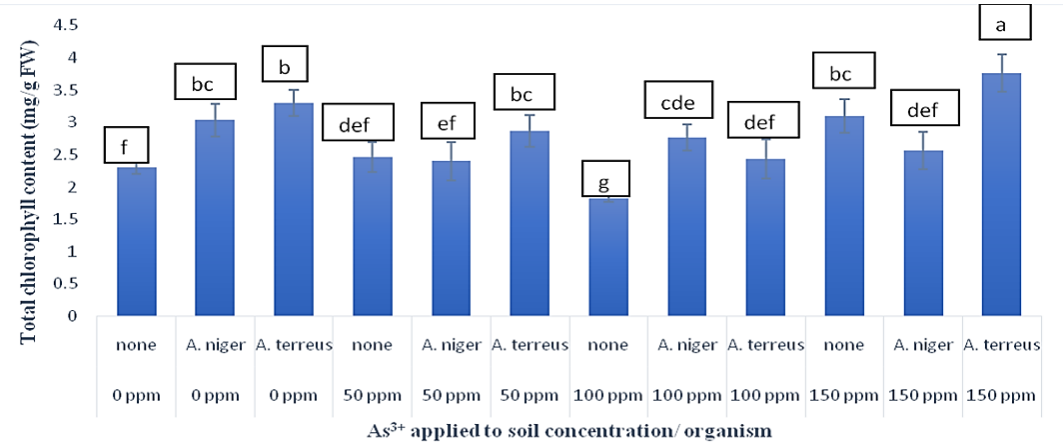

Figure 1. The total chlorophyll content in leaves of *Abelmoschus esculentus* plants grown in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*

Total carotenoids in leaves (Figure 2) also showed a positive effect with the presence of *A. niger* and *A. terreus*, in 0 ppm-100 ppm As^{3+} treated soils, showing 27.6% and 37.9% improvement in 50 ppm As^{3+}in *A. niger* and *A. terreus*, inoculated soils and 84.6% and 76.9% in 100 ppm As^{3+} in *A. niger* and *A. terreus*, inoculated soils respectively. Whereas at 150ppm As^{3+} treated soils, the plants grown in the presence of soil inocula, did not indicate positive contributions to the total carotenoids content of leaves.

The outcome of the analysis of superoxide dismutase (SOD) of plant tissues is shown in Figure 3. The SOD content of tissues of plants grown in 0.0 ppm As^{3+}, 50 ppm As^{3+}, 100ppm As^{3+} and 150 ppm As^{3+} soil sample without soil inoculants were observed to be significantly different. The values increased as the As^{3+} concentration applied to soil increased. Application of *Aspergillus niger* increased SOD content in tissues of plants grown 0 ppm and 150 ppm As^{3+}, whereas, decrease SOD content in 50 ppm and 100 ppm As^{3+} treated soils. Whereas *Aspergillus terreus* applied as soil inoculants decreased SOD content in all tissues of plants grown in As^{3+} treated soils. Higher depressions in SOD contents of tissues were recorded in 50 ppm and 100 ppm As^{3+} treated plants.

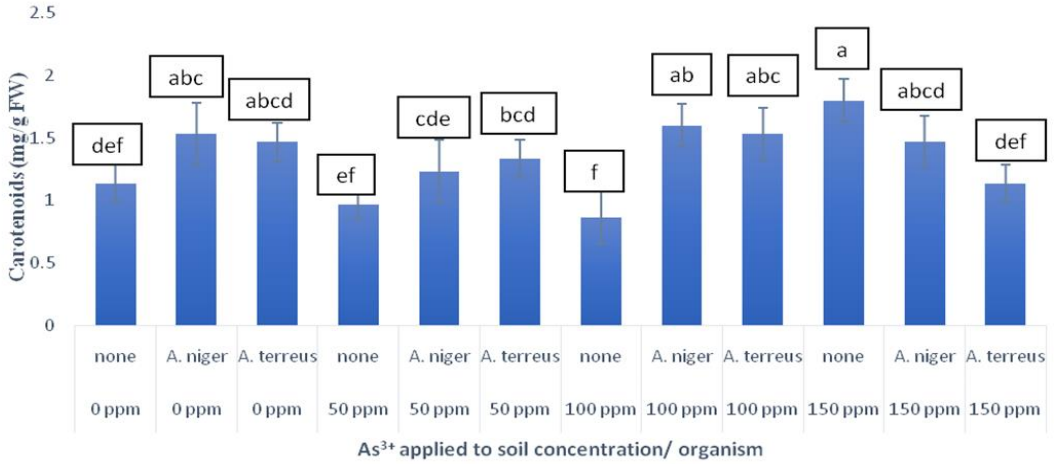

Figure 2. The quantity of carotenoids in leaves of *Abelmoschus esculentus* plants grown in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*

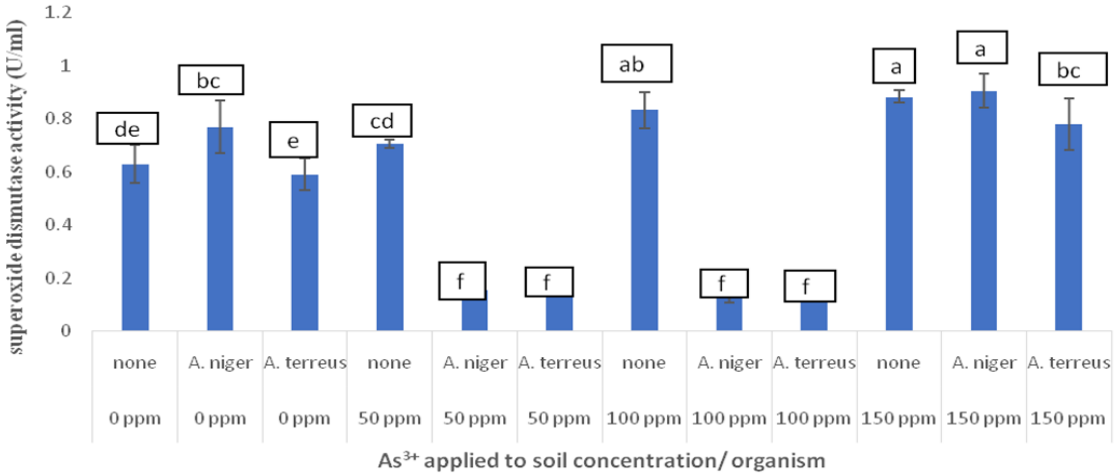

Figure 3. The superoxide dismutase activity in leaves of *Abelmoschus esculentus* plants grown in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*

The catalase activity analyzed in plant tissues is shown in Figure 4, where catalase contents in tissues decreases as the As^{3+} solutions applied to the soils and without inocula increased in concentration. Inoculation of 50 ppm, 100 ppm and 150 ppm As^{3+} treated soils with *A. terreus* improved catalase contents in tissues, showing 16.6%, 41.5% and 25.4% increases, respectively.

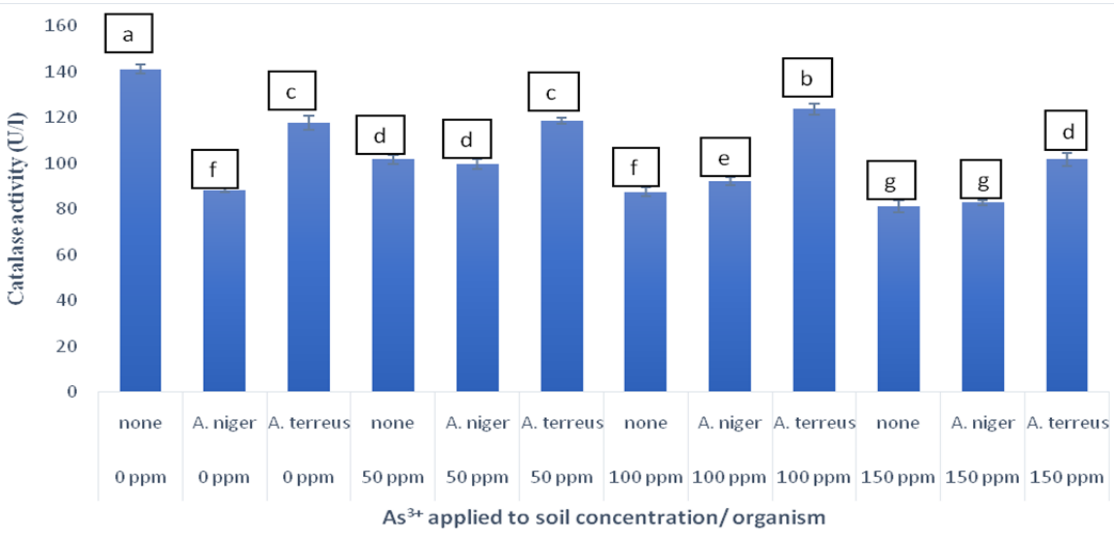

Figure 4. The catalase activity in leaves of *Abelmoschus esculentus* plants grown in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*

Nitrate reductase (NR) activity in roots and leaves tissues is shown in Figure 5. The least values for nitrate reductase action were recorded in plants without As^{3+} treatments. Differences between root and leaves were not significant. Higher values for nitrate reductase were recorded for plants grown in As^{3+} treated soils. NR activity are generally higher than those in roots. Plants grown in soils with inocula, showed increased NR activity across the different concentrations.

Soil phosphatase content is shown in Figure 6. The variations observed among the soil samples were minimal, whether the soil inoculants were present or absent. In As^{3+}- treated soils, phosphatase contents were higher in the presence of soil inocula depicting higher activity.

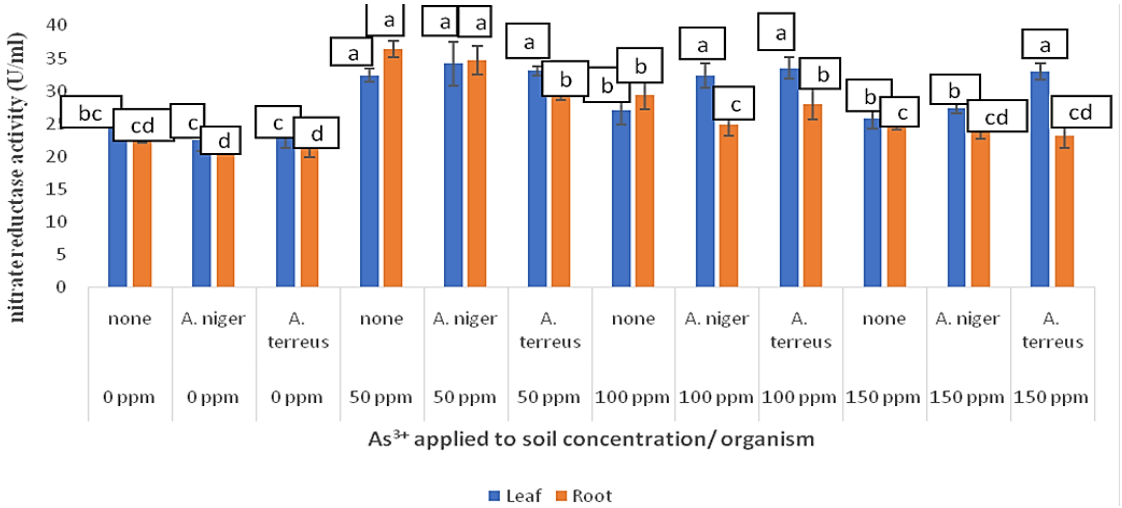

Figure 5. The nitrate reductase (NR) activity in leaves and roots of *Abelmoschus esculentus* plants grown in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*

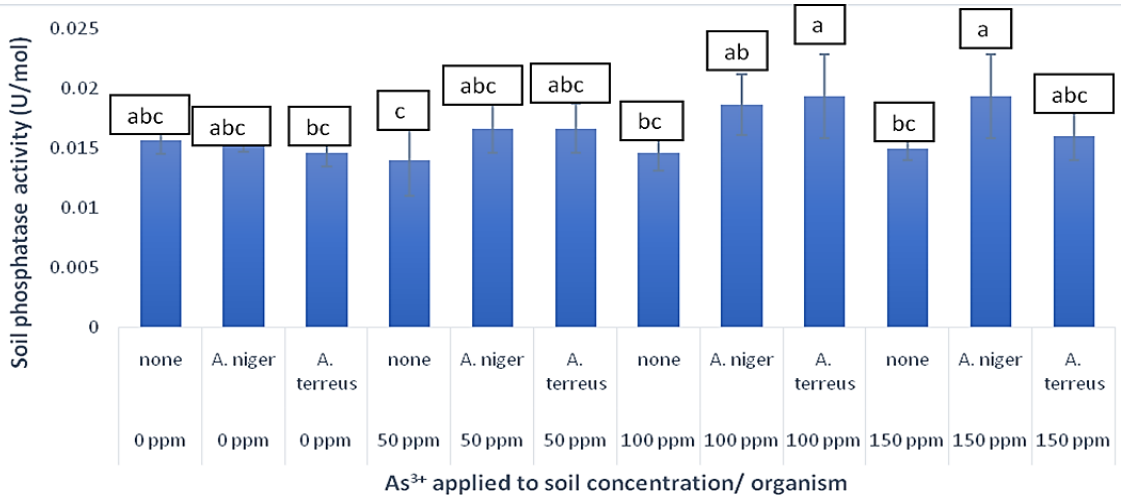

Figure 6. The soil phosphatase activity in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus* in which *Abelmoschus esculentus* plants were grown

Soil urease activity is shown in Figure 7. The addition of soil inocula did not improve urease activity in As^{3+} treated soils. Salient feature observed was that urease contents were suppressed in all As^{3+} treated soils augmented with *Aspergillus niger* as soil inoculant.

Soil cellulase activity is shown in Figure 8. The supplementation with soil inocula improved cellulase activity in As^{3+} treated soils. Higher cellulase contents were obtained in As^{3+} treated soils supplemented with soil inocula. The significant differences were observed between soils inoculated with organisms and those without organisms.

Soil dehydrogenase activity is shown in Figure 9. It was observed that the addition of soil inocula increase soil dehydrogenase contents marginally in all As^{3+} treated soils.

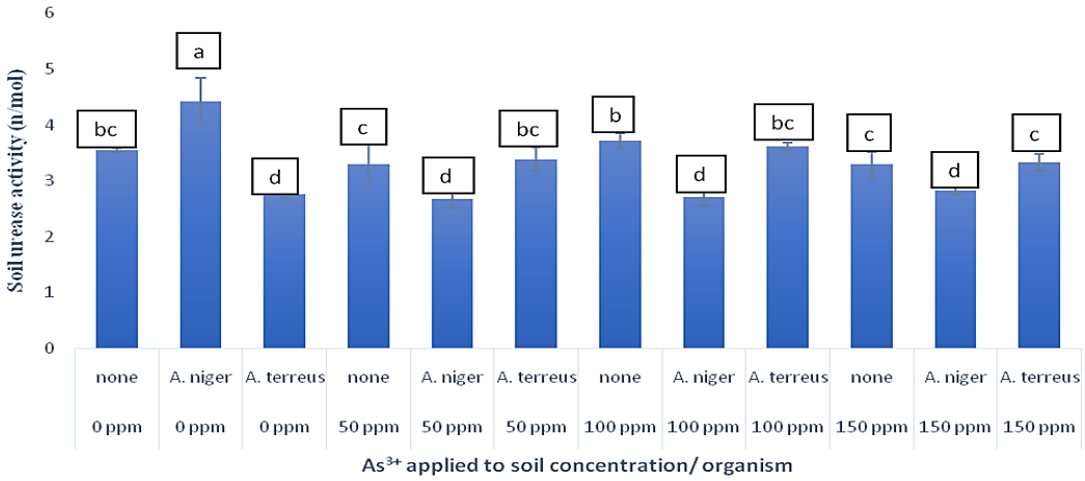

Figure 7. Soil urease activity in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus,* in which *Abelmoschus esculentus* plants were grown

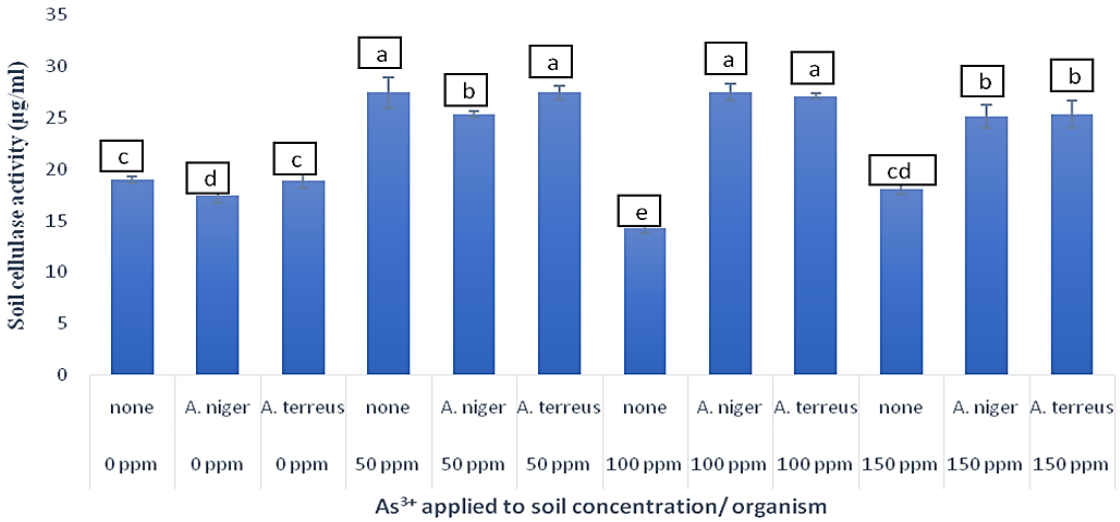

Figure 8. The soil cellulase activity in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*, in which *Abelmoschus esculentus* plants were grown

Discussion

In this study Fungi were isolated from heavy metal contaminated soils and grown on heavy metal supplemented PDA media. *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* showed the highest growth on PDA media. *Aspergillus fumigatus* could not survive storage after 6 weeks. Only *A. niger* and *A. terreus* were used as soil inoculants. Narendrula-Kotha and Nkongolo (2017) previously reported the presence of Ascomycota in heavy metal contaminated soils.

Arsenic is generally considered phytotoxic and is expected to negatively affect plant growth (Chaturvedi, 2006). Though the effects of the heavy metal applied to the soils in this work negatively affected the plants in the non-inoculated soils, while fungal inoculation improved growth of *Abelmoschus esculentus* plants as seen in the in increased in the number of leaves produced by the plants, increase in plant height and stem girth. Delayed flowering was observed in plants grown in soils treated with As^{3+}.

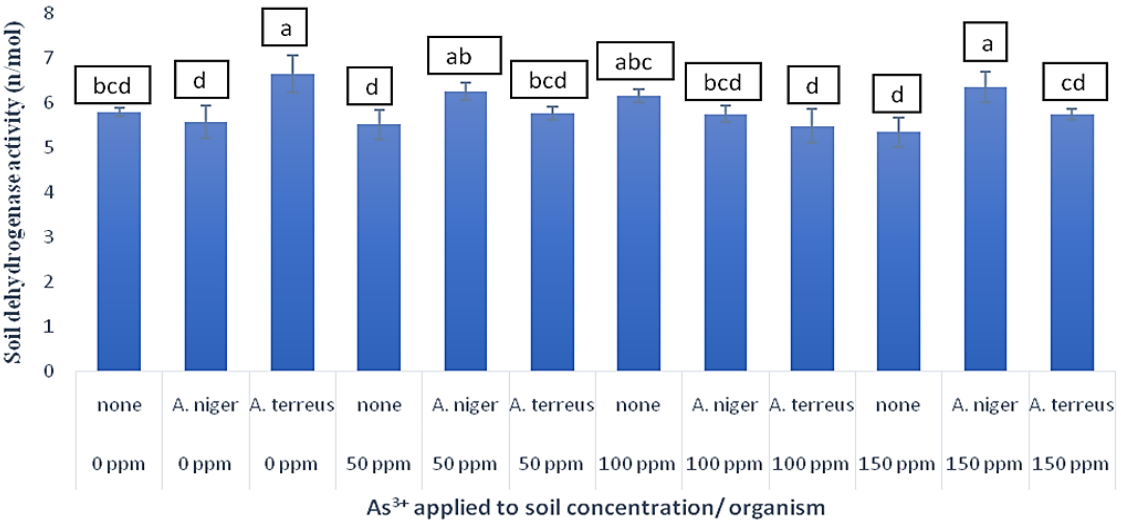

Figure 9. The soil dehydrogenase activity in soils treated with As^{3+} ion solutions and inoculated with *Aspergillus niger* and *Aspergillus terreus*, in which *Abelmoschus esculentus* plants were grown

Chlorophyll content is often measured to assess the impact of environmental stress in plants, as changes in chlorophyll content can associated with symptoms of plant disease and changes in photosynthetic productivity (Zengin and Munzuroglu, 2005). The accumulation of arsenic in the leaf biomass has been observed to reduce the rate of photosynthesis in plants (Marques and Anderson, 1986; Caporale *et al.,* 2014). It was observed from the results of this study that plants in soils inoculated with fungi showed increase in total chlorophyll content in most concentration of As^{3+} treated plants. This might be attributed to the ability of the fungi to reduce the stress caused by the presence of As^{3+} in the soil which improved chlorophyll synthesis by plants grown in As^{3+} contaminated soils. Also, soil inocula increased the quantity of carotenoids in plants grown in 50 ppm and 100 ppm As^{3+} treated soils.

In this regard, Kanwal *et al.* (2016) reported that in mycorrhizal associated wheat plants, the chlorophyll (a, b) and carotene contents were significantly higher in contrast with non-mycorrhizal associated wheat plants at all applied zinc concentrations (0, 100, 300 and 900 mg/kg). Caporale *et al.* (2014) reported that the inoculation of vetiver grass (*Chrysopogon zizaniodes* L.) with *Glomus spp.* significantly increased the amount of chlorophylls a and b in the leaf biomass of plants. Sharma *et al.* (2017) reported that the inoculation of arbuscular mycorrhizal fungi, led to an overall increase in the level of carotenoids as compared to the non-mycorrhizal inoculated plants.

The first defense against reactive oxygen species (ROS) in plants is usually superoxide dismutase, which acts on superoxide free radicals (Alscher and Erturk, 2002). This enzyme breaks down highly reactive superoxide to hydrogen peroxide, which is also another reactive oxidizing agent (Gunes *et al.*, 2009). It has been recently demonstrated that reactive oxygen species, superoxide dismutase and catalase are all induced in plants, after exposure to arsenic (Chaturvedi, 2006). This was also well observed in this study, as SOD activity increased with increasing concentration of As^{3+} in the leaves of plants grown in the non-inoculated soils. In a previous report by Issam *et al.* (2015) arsenic application increased SOD activity. Srivastava *et al.* (2005) also reported an increased SOD activity in response to arsenic toxicity in fern species. Similar findings were reported by Gunes *et al.* (2009) in maize and in the grass *Holcus lanatus* (Hartley-Whitaker *et al.*, 2001). The presence of fungi in soil may led to decreased SOD activities across the As^{3+} treated plants created an indication that fungi can provide assistance to plants in stressed conditions depending on the biochemical and physiological pathways adopted by the fungi.

From the present data it could be noted that at higher As^{3+} concentrations, the activity of catalase was decreased in plants grown in non-inoculated soils. Decreased activity of catalase with increasing heavy metal concentrations was previously reported for *Becopamonneri* (Mishra *et al.*, 2006) and *Lemnagibba* (Parlak and Yilmaz, 2013). It is possible that over production of ROS by heavy metal-induced stress can inactivate CAT activity at higher concentrations of the heavy metal ions, probably by inactivating the enzyme-bound heme group, as suggested by Malar *et al.* (2015). *Aspergillus terreus* was revealed by data from this study to increase CAT activity in As^{3+} stressed plants. Kanwal *et al.* (2016) reported that the contents of SOD were high in plants with mycorrhizal associated treatments, when compared to the non-mycorrhizal associated treatments. They also reported that CAT activity improved as the concentration of Zn increased in both non-mycorrhizal and mycorrhizal treated plants. At higher Zn application level (900 mg/kg), they observed decreased CAT activity.

Nitrate reductase is very sensitive to metal stress (Kumar and Joshi, 2008), as it is the rate-limiting enzyme in nitrogen assimilation (Singh *et al.*, 2009). NR activity in the leaves increased with the addition of heavy metals, and further increases was generated by the inoculation of fungi. In the roots, NR activity was observed to decrease in soils inoculated with fungi across As^{3+} treatments. Generally, NR activities of plants grown in As^{3+} treated soils were observed to be higher in the leaves than the root. In contrast to this study, Singh *et al.* (2009) reported a marked inhibition of NR activity by arsenic in *P. ensiformis*. The ability of these fungi organisms to enhance the tolerance of *Abelmoschus esculentus* to arsenic stress can be linked to their ability

to restrict arsenic translocation from roots to shoots of plants as suggested by Gonzalez Chavez *et al.* (2002), Chen *et al.* (2007) and Ultra *et al.* (2007).

Soil enzymes are considered as useful indicators of soil quality, in regards to soil contamination and pollution. Soil enzymes play important roles in the chemical changes that occur in the soil, involving soil nutrients (Dike *et al.*, 2013). Different soil enzymes exhibit different level of sensitivities to different heavy metals. Shen *et al.* (2005) reported that urease and dehydrogenase could be appropriate indicators of pollution, especially at the initial stages. Soil enzyme activities are influenced in different ways by different metals due to the different chemical affinities of the enzymes in the soil system. Phosphatase and urease are involved in the biochemical cycles of phosphorus and nitrogen respectively (Li *et al.*, 2005). In this study, the fungi organisms were observed to stimulate or increase soil cellulase and phosphatase activities across the As^{3+} treated soil. Sethi and Gupta (2015) stated that soil dehydrogenase activity was found to be inhibited after the application of heavy metals. Nweke *et al.* (2007) also concluded that for all the metal ions, there was progressive inhibition in dehydrogenase activity with increase in the concentration of metal ions. These reports are in agreement with the present data from this work, where dehydrogenase activity was inhibited by application of heavy metals. However, soils inoculated with fungi organisms showed high dehydrogenase activity than the non-inoculated soils.

Conclusions

This study might provide a useful information on the potentials of fungi inocula as important tools for alleviating heavy metal stress in Okra plants. The study emphasised the ameliorative effect of fungi inoculation in enhancing plant growth in arsenic contaminated soils, as seen in the increase in plant height, number of leaves and in antioxidant activities. Soil cellulase activity was highly improved by fungi inoculants, giving an insight that fungi inocula can be used for soil improvements. Therefore, the findings of this study reveal that the use of fungi inoculants would be important tools to improve plant arsenic resilience.

Authors' Contributions

The MO executed the field experiments, wrote the original draft and analysed the data. TAD executed the mycological experiments and helped in the preparation of the manuscript. EDV designed the work, interpreted the data, reviewed and edited the main manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abedin MJ, Feldmann J, Meharg AA (2002). Uptake kinetics of arsenic species in rice plants. Plant Physiology 128:1120-1128. *https://doi.org/10.1021/es0101678*

Abidi B, Singha P, Chauhana V, Tiwaria, BK Chauhan SS, Simonb S (2014). An overview on Okra (*Abelmoschus esculentus*) andits importance as a nutritive vegetable in the world. International Journal of Pharmacy and Biological Sciences 4(2):227-233. *https://ijpbs.com/ijpbsadmin/upload/ijpbs_53df5a2907b19.pdf*

Ahsan N, Lee DG, Alam I, Kim PJ, Lee JJ, Ahn YO, ... Lee BH (2008). Comparative proteomic study of arsenic-induced differentially expressed proteins in rice roots reveals glutathione plays a central role during As stress. Proteomics 8:3561-3576. *https://doi.org/10.1002/pmic.200701189*

Akinyele, BO, Temikotan T (2007). Effect of variation in soil texture on the vegetative and pod characteristics of okra (*Abelmoschus esculentus*(L) Moench). International Journal of Agricultural Research 2:165-169. *https://doi.org/10.3923/ijar.2007.165.169*

Alika JE (2006). Research Methods and Statistics. AMBIL press, Benin City, Nigeria, 336p.

Alscher RG, Erturk NL (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Environmental and Experimental Botany 53:1331-1341. *http://dx.doi.org/10.1093/jexbot/53.372.1331*

Andras CD, Simandi B, Orsi F, Lambrou C, Missopolinou-Tatala D, Panayiotou C, ... Doleschall F (2005). Supercritical carbon dioxide extraction of okra (*Hibiscus esculentus* L) seeds. Journal of the Science of Food and Agriculture 85(8):1415-1419. *https://doi.org/10.1002/jsfa.2130*

Arnon DI (1949). Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24 (1):1-15. *https://doi.org/10.1104/pp.24.1.1*

Barnett HL, Barry BH (1998). Illustrated Genera of Imperfect Fungi. 4th (fourth) Edition. Amer Phyto-pathological Society. Pp 218.

Broadbent FE, HILL CN, Tyler KB (1958). Transformations and movement of urea in soils. Soil Science Society of America Proceedings 22:303-307. *https://doi.org/10.2136/sssaj1958*

Burlo F, Guijarro I, Carbonell-Barrachina AA, Valero D, Martinez-Sanchez F (1999). Arsenic species: effects on and accumulation by tomato plants. Journal of Agriculture and Food Chemistry 47:1247-1253. *https://doi.org/10.1021/jf9806560*

Caporale AG, Sarkar D, Datta R, Punamiya P, Violante A (2014). Effect of arbuscular mycorrhizal fungi (*Glomus* spp) on growth and arsenic uptake of vetiver grass (*Chrysopogon zizanioides* L) from contaminated soil and water systems. Journal of Soil Science and Plant Nutrition 14(4):955-972. *https://doi.org/10.4067/S0718-95162014005000075*

Carbonell-Barrachina AA, Burlo F, Valero D, Lopez E, Martinez-Romero D, Martinez-Sanchez F (1999). Arsenic toxicity and accumulation in turnip as affected by arsenical chemical speciation. Journal of Agriculture and Food Chemistry 47:2288-2294. *https://doi.org/10.1021/jf981040d*

Casida LE, Klein DA, Santoro T (1964). Soil dehydrogenase activity. Soil Science 98(6):371-376. *https://doi.org/10.1097/00010694-196412000-00004*

Chandra S, Saha R, Pal P (2016). Arsenic uptake and accumulation in okra (*Abelmoschus esculentus*) as affected by different arsenical speciation. Bulletin of Environmental Contamination and Toxicology 96:395-400. *https://doi.org/10.1007/s00128-015-1712-4*

Chaturvedi I (2006). Effects of arsenic concentrations and forms on growth and arsenicuptake and accumulation by Indian mustard (*Brassica juncea* L) genotypes. Journal of Central European Agriculture 7(1):125-138. *https://hrcak.srce.hr/17339*

Chen BD, Xiao XY, Zhu YG, Smith FA, Xie ZM, Smith SE (2007). The arbuscular mycorrhizal fungus *Glomus mosseae*gives contradictory effects on phosphorus and arsenic acquisition by *Medicago sativa* Linn. Science of the Total Environment 379:226-234. *https://doi.org/10.1016/j.scitotenv.2006.07.038*

Deng Z, Cao L, Huang H, Jiang X, Wang W, Shi Y, Zhang R (2011). Characterization of Cd- and Pb-resistant fungal endophyte *Mucor sp* CBRF59 isolated from rapes (*Brassica chinensis*) in a metal-contaminated soil. Journal of Hazardous Materials 185:717-724. *https://doi.org/10.1016/j.jhazmat.2010.09.078*

Dike CC, Ofoegbu CJ, Akubugwo EI, Maduka HCC, Ugwu CE, Obasi NA (2013). Effects of heavy metals on soil enzymatic activities in theIshiagu mining area of Ebonyi State, Nigeria. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 5(6):2319-2402. *https://doi.org/10.9790/2402-0566671*

Doumett S, Lamperi L, Checchini L, Azzarello E, Mugnai S, Mancuso S, Petruzzelli G, Del Bubba M (2008). Heavy metal distribution between contaminated soil and *Paulownia tomentosa*, in a pilot-scale assisted phytoremediation study: influence of different complexing agents. Chemosphere 72:1481-1490. *https://doi.org/10.1016/j.chemosphere.2008.04.083*

Eivazi F, Tabatabai MA (1977). Phosphatases in soils. Soil, Biology and Biochemistry 9:167-172. *https://doi.org/10.1016/0038-0717(77)90070-0*

Ellis MB, Ellis JP (1997). Microfungi on Land Plants: An Identification Handbook. Richmond Publishers, Slough, England. Pp 868.

Garg N, Singla, P (2011). Arsenic toxicity in crop plants: Physiological effects and tolerance mechanisms. Environmental Chemistry Letters 9:303-321. *https://doi.org/10.1007/s10311-011-0313-7*

Gonzalez-Chavez MC, Harris PJ, Dodd J, Meharg AA (2002). Arbuscular mycorrhizal fungi enhanced arsenate resistance on *Holcus lanatus*. New Phytologist155:163-171. *https://doi.org/10.1046/j.1469-8137.2002.00430.x*

Gross J (1991). Pigments in Vegetables, Chlorophylls and Carotenoids. Van Nostrand Reinhold, New York, pp 351. *https://doiorg/101007/978-1-4615-2033-7*

Gunes A, Pilbeam D, Inal A (2009). Effect of arsenic-phosphorusinteraction on arsenic-induced oxidative stress in chickpea plants. Plant and Soil 314(1-2):211-220. *https://doi.org/10.1007/s11104-008-9719-9*

Gusman GS, Oliveira JA, Farnese FS, Cambraia J (2013). Mineral nutrition and enzymatic adaptation induced by arsenate and arsenite exposure in lettuce plants. Plant Physiology and Biochemistry 71:307-314. *https://doi.org/10.1016/j.plaphy.2013.08.006*

GWRTAC (1997). Remediation of metals-contaminated soils and groundwater. Tech Rep TE-97-01, GWRTAC, Pittsburgh, Pa, USA, GWRTAC-E Series. *https://clu-in.org/download/toolkit/metals.pdf*

Hageman RH, Hucklesby DP (1971). Nitrate reductase In: Methods in Enzymology 23:491-503. *https://doi.org/10.1016/S0076-6879(71)23121-9*

Hartley-Whitaker J, Ainsworth G, Meharg AA (2001). Copper and arsenate-induced oxidative stress in *Holcus lanatus*L clones with differential sensitivity. Plant Cell and Environment 24:713-722. *https://doi.org/10.1046/j.0016-8025.2001.00721.x*

Ike A, Sriprang R, Ono H, Murooka Y, Yamashita M (2007). Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant rhizobia with the MTL4 and the PCS genes. Chemosphere 66:1670-1676. *https://doi.org/10.1016/j.chemosphere.2006.07.058*

Issam S, Nawel N, Yassine C (2015). Selenium alleviates the arsenic toxicity in sunflower seedling. Journal of Advances in Biology and Biotechnology 3(3):101-109. *https://doi.org/10.9734/JABB/2015/17243*

Jiang QY, Zhuo F, Long SH, Zhao HD, Yang DJ, Ye ZH, (2016). Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of *Lonicera japonica*grown in Cd added soils? Scientific Reports 6:21805. *https://doi.org/10.1038/srep21805*

Kanwal S, Bano A, Malik, RN (2016). Role of arbuscular mycorrhizal fungi inphytoremediation of heavy metals and effects ongrowth and biochemical activities of wheat (*Triticum aestivum* L) plants in Zn contaminated soils. African Journal of Biotechnology 15(20):872-883. *https://doi.org/10.5897/AJB2016.15292*

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197-207. *https://doi.org/101016/S0045-6535(99)00412-9*

Khan AL, Lee IJ (2013). Endophytic *Penicillium funiculosum* LHL06 secretes gibberellin that reprograms *Glycine max* L growth during copper stress. BMC Plant Biology 13:86. *https://doi.org/10.1186/1471-2229-13-86*

Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environmental Pollution 152:686-692. *https://doi.org/10.1016/j.envpol.2007.06.056*

Koroliuk MA, Ivanova LI, Maĭorova IG, Tokarev VE (1988). A method of determining catalase activity. Lab Delo 1:16-19.

Kumar S, Joshi UN (2008). Nitrogen metabolism as affected by hexavalent chromium in sorghum (*Sorghum bicolor* L). Environmental and Experimental Botany 64:135-144. *https://doi.org/10.1016/j.envexpbot.2008.02.005*

Li H, Zhang Y, Zhang CG, Chen GX (2005). Effect of Petroleum-containing wastewater irrigation on bacteria diversities and enzymatic activities in a paddy soil irrigation area. Journal of Environmental Quality 34:1073-1080. *https://doi.org/10.2134/jeq2004.0438*

Liu X, Zhang S, Shan X, Zhu YG (2005). Toxicity of arsenate and arsenite on germination seedling growth and amylolytic activity of wheat. Chemosphere 61:293-301. *https://doi.org/10.1016/j.chemosphere.2005.01.088*

Malar S, Sahi SV, Favas PJC, Venkatachalam P (2015). Assessment of mercury heavy metal toxicity-induced physiochemical and molecular changes in *Sesbania grandiflora* L. International Journal of Environmental Science and Technology 12:3273-3282. *https://doi.org/10.1007/s13762-014-0699-4*

Mallick S, Sinam G, Sinha S (2011). Study on arsenate tolerant and sensitive cultivars of *Zea mays* L: Differential detoxification mechanism and effect on nutrients status. Ecotoxicology and Environmental Safety 74:1316-1324. *https://doi.org/10.1016/j.ecoenv.2011.02.012*

Marques IA, Anderson LE (1986). Effects of arsenite, sulphite, and sulphate on photosynthetic carbon metabolism in isolated pea (*Pisum sativum* L. cv. Little Marvel) chloroplasts. Plant Physiology 82:488-493. *https://doi.org/10.1104/pp.82.2.488*

Massaccesi G, Romero MC, Cazau MC, Bucsinszky AM (2002). Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, in La Plata (Argentina). World Journal of Microbiology and Biotechnology 18:817-820. *https://doi.org/10.1023/A:1021282718440*

Mishra S, Srivastava S, Tripathi RD, Govindarajan R, Kuriakose SV, Prasad MNV (2006). Phytochelation synthesis and responses of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiology and Biochemistry 44:25-37. *https://doi.org/10.1016/j.plaphy.2006.01.007*

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247:3170–3175. *https://doi.org/10.1016/S0021-9258(19)45228-9*

Moller IM, Jensen PE, Hansson A (2007). Oxidative modifications to cellular components in plants. Annual Review of Plant Biology 58:459-481. *https://doi.org/10.1146/annurev.arplant.58.032806.103946*

Morton-Bermea O, Hernandez-Alvarez E, Gaso I, Segovia N (2002). Heavy metal concentrations in surface soil from Mexico City. Bulletin of Environmental Contamination and Toxicology 68: 383-388. *https://doi.org/10.1007/s001280265*

Nagajyoti P, Lee K, Sreekanth T (2010). Heavy metals, occurrence and toxicity for plants: A review. Environmental Chemistry Letters 8:199-216. *http://dx.doi.org/10.1007/s10311-010-0297-8*

Narendrula-Kotha R, Nkongolo KK (2017). Microbial response to soil liming of damaged ecosystems revealed by pyrosequencing and phospholipid fatty acid analyses. PLoS One 12(1):e0168497 *https://doi.org/101371/journalpone0168497*

Nel A, Xia T, Mädler L, Li N (2006). Toxic potential of materials at the nano level. Science 311:622-627. *https://doi.org/10.1126/science.1114397*

Nweke CO, Ntinugwa V, Obah IF, Ike SC, Eme GE, Opara EC, Okolo JC, Nwanyanwu V (2007). In vitro effects of metals and pesticides on dehydrogenase Activity in microbial community of cowpea (*Vigna unguiculata*) rhizoplane. African Journal of Biotechnology 6:290-295. *https://doi.org/10.5897/AJB06.680*

Pancholy SK, Rice EL (1973). Soil enzymes in relation to old field succession: Amylase, cellulase, invertase, dehydrogenase and urease. Soil Science Society of America Proceedings 37:47-50.

Parlak KC, Yilmaz DD (2013). Ecophysiological tolerance of *Lemnagibba* L exposed to cadmium. Ecotoxicology and Environmental Safety 91:79-85. *https://doi.org/10.1016/j.ecoenv.2013.01.009*

Punamiya P, Datta R, Sarkar D, Barber S, Patel M, Das P (2010). Symbiotic role of *Glomus mosseae* in phytoextraction of lead in vetiver grass [*Chrysopogon zizanioides* (L)]. Journal of Hazardous Materials 177:465-474. *https://doi.org/10.1016/j.jhazmat.2009.12.056*

Ren G, Chen Y, Zou XK, Zhou YQ (2011). Change in climatic extremes over mainland China based on an integrated extreme climate index. Climate Research 50:113-124. *https://doi.org/10.3354/cr01023*

Salehi A, Kouchaksaraei MT, Goltapeh EM, Shirvany A, Mirzaei J (2016). Effect of mycorrhizal inoculation on black and white poplar in a lead-polluted soil. Journal of Forest Science 62(5):223-228. *https://doi.org/1017221/23/2016-JFS*

Sethi S, Gupta S (2015). Responses of soil enzymes to different heavy metals. Biolife 3(1):147-153. *https://www.academia.edu/21760831/Responses_of_soil_enzymes_to_different_heavy_metals*

Shahid M, Dumat C, Pourrut B, Abbas G, Shahid N, Pinelli E (2015). Role of metal speciation in lead-induced oxidative stress to *Vicia faba* roots. Russian Journal of Plant Physiology62:448-454. *https://doi.org/10.1134/S1021443715040159*

Sharma S, Anand G, Singh N, Kapoor R (2017). Arbuscular mycorrhiza augments arsenic tolerance in wheat (*Triticum aestivum* L) by strengthening antioxidant defense system and thiol metabolism. Frontiers of Plant Science 8:906 *https://doi.org/103389/fpls201700906*

Shen G, Lu Y, Zhou Q, Hang J (2005). Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme. Chemosphere 61:1175-1182. *https://doi.org/10.1016/j.chemosphere.2005.02.074*

Singh N, Lena Q, Ma A, Joseph C, Vu B, Anshita R (2009). Effects of arsenic on nitrate metabolism in arsenic hyperaccumulating and non-hyperaccumulating ferns. Environmental Pollution 157:2300-2305. *https://doi.org/10.1016/j.envpol.2009.03.036*

Singh N, Ma LQ, Srivastava M, Rathinasabapathi B (2006). Metabolic adaptations to arsenic-induced oxidative stress in *Pteris vittata L* and *Pteris ensiformis L*. Plant Science 170:274-282. *https://doi.org/10.1016/j.plantsci.2005.08.013*

Soleimani M, Hajabbasi MA, Afyuni M, Mirlohi A, Borggaard OK, Holm PE (2010). Effect of endophytic fungi on cadmium tolerance and bioaccumulation by *Festuca arundinacea* and *Festuca pratensis*. International Journal of Phytoremediation 12:535-549. *https://doi.org/10.1080/15226510903353187*

Srivastava M, Ma LQ, Singh N, Singh S (2005). Antioxidant responses of hyper-accumulator and sensitive fern species to arsenic. Journal of Experimental Botany 56:1332-1342. *https://doi.org/10.1093/jxb/eri134*

St-Germain, G, Summerbell R (1996). Identifying Filamentous Fungi: A Clinical Laboratory Handbook. Star Publishing Company, Singapore. *https://doi.org/10.1590/s0036-46652003000300017*

Turner NC (1981). Techniques and experimental approaches for the measurement of plant water status. Plant and Soil 58:339-366. *https://doi.org/10.1007/BF02180062*

Ultra VU, Tanaka S, Sakurai K, Iwasaki K (2007). Effects of arbuscular mycorrhiza and phosphorus application on arsenic toxicity in sunflower (*Helianthus annuus*L) and on the transformation of arsenic in the rhizosphere. Plant and Soil 290:29-41. *https://doi.org/10.1007/s11104-006-9087-2*

USEPA (1996). Report: recent developments for *in situ* treatment of metals contaminated soils. US Environmental Protection Agency, Office of Solid Waste and Emergency Response. *https://www.epa.gov/sites/default/files/2015-08/documents/recent_dev_metals_542-r-97-004.pdf*

Vwioko DE, Edobor FO (2017). Comparing the phytotoxicity effects of As III and As V ionic soil treatments on the growth of *Cyperus esculentus* (L) tubers. Ege University Journal of the Faculty of Science 41:14-31.

Waksman SA (1927). Principles of soil microbiology. William and Wilkins, Batimore. *https://doi.org/10.5962/bhl.title.7930*

Xiao X, Luo S, Zeng G, Wei W, Wan Y, Chen L, ... Xi Q (2010). Biosorption of cadmium by endophytic fungus (EF) *Microsphaeropsissp* LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L. Bioresource Technology 101:1668-1674. *https://doi.org/10.1016/j.biortech.2009.09.083*

Zengin FK, Munzuroglu O (2005). Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L) seedlings. Acta Biologica Cracoviensia Series Botanica 47(2):157-164. *https://abcbot.pl/pdf/47_2/157-164.pdf*

Zhang D, Duine JA, Kawai F (2002). The extremely high Al resistance of *Penicillium janthinellum* F-13 is not caused by internal or external sequestration of Al. Biometals 15:167-174. *https://doi.org/10.1023/a:1015289808484*

Zhang MK, Liu ZY, Wang H (2010). Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. Communications in Soil Science and Plant Analysis 7:820-831. *https://doi.org/10.1080/00103621003592341*