Journal of Research in Biology

An International Scientific Research Journal

ISSN No: Print: 2231 – 6280; Online: 2231- 6299.

Original Research

Associations of Arbuscular Mycorrhizal (AM) fungi in the Phytoremediation of Trace Metal (TM) Contaminated Soils.

Authors:
Dhritiman Chanda [1], Sharma GD[2], Jha DK[3] and Hijri M[4].

Institution:
1. Microbiology Laboratory, Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India.

2. Bilaspur University, Bilaspur, India.

3. Department of Botany, Gauhati University, Assam, India.

4. Institut de Recherche en Biologie Vegetale, University de Montreal, Montreal, Canada.

Corresponding author:
Dhritiman Chanda.

ABSTRACT:

Arbuscular mycorrhizal fungi (AM) are integral, functioning parts of plant roots, widely recognized as plant growth enhancing beneficial mycobionts and tolerance to variety of stresses such as nutrient, drought, salinity and trace metals (TM). A study was undertaken to access the influence of paper mill effluents on mycorrhizal colonization and mycorrhizal spore count. Plants grown in metal contaminated site were found less mycotrophic than their counterparts on the non-polluted one. Regression analyses revealed that the mycorrhizal colonization and mycorrhizal spore count are significantly and positively correlated with various soil physio-chemical properties in the polluted and non-polluted site. *Glomus* was the most frequently isolated mycorrhizal species from the polluted site. The isolated indigenous strains of AM can be used for inoculation of plant species that might be used for rehabilitation of contaminated site. The study highlights the potential use of AM as bioremediation agent of polluted soils and as bioindicator of pollution for future research priorities.

Keywords:
Arbuscular Mycorrhiza, Heavy metals, Phytoremediation, Glomus, Paper mill effluents.

Email Id:
chandadhriti@gmail.com

Web Address:
http://jresearchbiology.com/documents/RA0416.pdf.

Article Citation:
Dhritiman Chanda, Sharma GD, Jha DK and Hijri M.
Associations of Arbuscular Mycorrhizal (AM) fungi in the Phytoremediation of Trace Metal (TM) Contaminated Soils.
Journal of Research in Biology (2014) 4(2): 1247-1263

Dates:
Received: 17 Jan 2014 **Accepted:** 22 March 2014 **Published:** 23 April 2014

INTRODUCTION:

Arbuscualr mycorrhizal (AM) fungi are ubiquitous obligate mycobionts forming symbiosis with the terrstrial plant communities (Barea and Jeffries 1995). They are essential components of soil biota and are found in almost all ecological situations particularly those supporting plant communities with high species diversity. AM are known to enhance plant tolerance to a variety of stresses including nutrients, drought, metal toxicity, salinity and pathogens all of which may affect plants success in a contaminated or polluted soil (Olexa *et al.,* 2000; Zarei *et al.,* 2010). AM can help alleviate metal toxicity to plants by reducing metal translocation from root to shoot (Leyval *et al.,* 1997). Therefore they may contribute to plant establishment and survival in trace metals polluted sites and could be used as a complement to immobilization strategies. In the last few years, research interest has been focused on the diversity and tolerance of AM in trace metals contaminated soil. To understand the basis underlying adaptation and tolerance of AM to trace metals in soils,since this could facilitate and manage these soil microoraganisms for restoration and bioremediation programs (Khan *et al*., 2000; Shah *et al*., 2010). AM constitute an important functional component of the soil plant system that is critical for sustainable productivity in stressed soils and promote plant growth to reduce or eliminate the bioavilibility of plants as studied by Joner and Leyval (2003). The variation in metal accumulation and inter-plant translocation depends on the different factors like host-plant, root density, soil characteristics, metals and their availibility. Metal tolerant AM isolates can decrease metal absorption capacity of these fungi, which could filter metal ions during uptake as described (Val *et al*., 1999; Andrew *et al*., 2013 and Martina and Vosatka (2005)). AM increases its host's uptake of nutrients and can improve the growth and resistance to environmental stresses (Biro *et al*., 2005; Smith and Read, 2008).

AM fungi could prove beneficial in phytoremediation system as they can increase the rate of plant survival and establishment, reduce plant stress and increase plant nutrients acquisition, increase carbon and nitrogen deposition into soil, thereby contributing to bacterial growth and increase the volume of soil being remediated (Almas *et al*., 2004).Trace metals concentration may decrease the number and vitality of AM as a result of HM toxicity. Metal transporters and plant-encoded transporters are involved in the tolerance and uptake of TM (Glassman and Casper 2012; Rahmanian *et al*., 2011).

In recent times, one of the challenges facing the mankind is the degradation and pollution of soil by industrial effluents, sludge and solid waste. The pulp and paper mill which has been categorized as one of the twenty most polluting industries in India discharge huge quantities of coloured and waste water (effluent) into the environment and are responsible for soil pollution consequently the hazardous chemicals enter into surface or ground water and poison the soil or crops. The decline of plant diversity is due to the soil toxicity generated by dumping of solid paper mill wastes in the area. Several researches have been carrying out to understand the role of AM fungi in plant interaction with toxic metal for promoting plant growth and the bioavailibility in stressed soils. In order to develop the restoration protocol for disturbed habitats, it is necesaary to study benificial rhizosphere fungi like AM fungi that are tolerant to various stresses. This will help us develop a protocol by studying the association of arbuscular mycorrhizal fungi in plants growing in soils polluted with paper mill effluents.

MATERIAL AND METHODS:

Location of the study area:

The study was conducted at two sites i.e. one polluted with paper mill effluents and another non-polluted site. The first site was effluent dumping site

inside the campus of Hindustan Paper Corporation Limited, HPC, Assam, India. The two sites were approximately 2 Km apart. The study area was located at an altitude of 116mMSL between 24052`N and 92036`E longitides.

Collection of soil Sample:

From the polluted and non-polluted site,10 dominant plant species were selected for the study of mycorrhizal association. The rhizosphere soil samples of these individuals of a species were collected. The rhizospheric soil samples were randomly selected and then mixed together to obtain a composite representative sample. The soil samplings were done trimonthly from April 2010 to January 2012. The soil samples were brought to the laboratory in sterile condition and stored in a refrigerator at 4°C until they were processed.

Collection of root samples:

Fine roots from ten dominant different plants of the same species were randomly collected and mixed properly and a composite root sample was obtained for each plant species. Trypan blue method was followed for the determination of the intensity of root colonization as described by Phillips and Hayman (1970).

Isolation of Mycorrhizal spores:

Spore extraction from the soil was carried out using the Wet Sieving and Decanting Technique by Gerdemann and Nicolson (1963). The isolated spores were mounted on glass slide using Polyvinyl Alcohol-Lactic acid Glycerol (PVLG) and observed under compound microscope (100-1000X). Spores were identified according to the manual of identification of VAM fungi by Schenck and Perez (1990). The INVAM worksheet was used for diagnosing the spores. Additional spores not included in the manual were identified as per the description given in the INVAM website (http://invam.caf.wvu.edu/).

Soil Physico-chemical analysis:

The physical chracteristics of soil i.e., Moisture content, soil pH and soil temperature were recorded in both polluted and non-polluted sites.

The chemical chracteristic i.e., N, P, K, Ca, Mg etc of the soil samples were estimated using the technique in the polluted and non-polluted site (Jackson,1985). Concentration of trace metalss i.e., Zn,

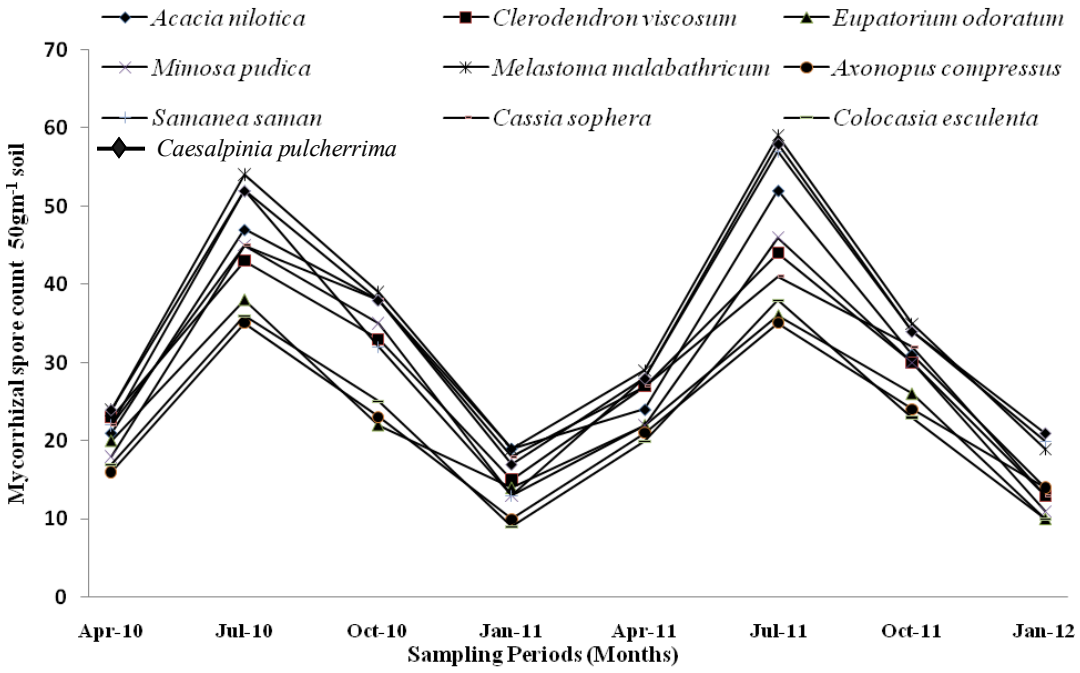


Fig 1: Monthly variation in Mycorrhizal spore population 50gm^{-1}soil of different plant species growing in the polluted site.

Ni and Cu were determined by Atomic Absorption Spectrophotometer (VARIAN Spectra AA 220).

Statistical analysis:

Statistical analysis was carried out by following the techniques of Gomez and Gomez (1984). Linear Regression analyses and correlation-coefficient values were calculated to find out the influence and association of various edaphic factors with mycorrhizal spore population and mycorrhizal root colonization (%) in the both polluted and non-polluted site.

RESULTS AND DISCUSSION:

The plants were more mycotrophic in the non-polluted site than those growing in the polluted site. The maximum root colonization was obtained in July both in the polluted and non-polluted site. The mycorrhizal root colonization were estimated maximum in the month of July and decreased gradually from October to January and again increased from April. The rhizosphere soil of the non-polluted site harboured more mycorrhizal spores in all the selected plants than the non-polluted site. Among the different plant species studied, maximum mycorrhizal spore count was estimated in *Melastoma malabathricum* (54, 50 gm^{-1} soil) followed by *Samanea saman* (52, 50 gm^{-1} soil) and *Caesalpinia pulcherrima* (49, 50 gm^{-1} soil) in the polluted site and in the non-polluted site *Melastoma malabathricum* (123, 50 gm^{-1} soil) harboured maximum number of mycorrhizal spores followed by *Samanea saman* (109,50 gm^{-1} soil) ,*Cassia sophera* (109,50 gm^{-1} soil) and *Caesalpinia pulcherrima* (98, 50 gm^{-1} soil) (Figures-1 and 2).

The maximum root colonization was obtained in July and found decreased gradually until January and again increased in April studied among the different plant species studied in the both polluted and non-polluted site. In the polluted site the maximum root colonization was estimated in *Melastoma malabathricum* (44%) followed by *Caesalpinia pulcherrima* (43%) and *Mimosa pudica* (41%) and the minimum percentage colonization was obtained in *Colocasia esculenta* (35%) and *Axonopus compressus* (32%). In the non-polluted site the maximum root colonization was estimated in *Melastoma malabathricum* (68%) followed by *Caesalpinia pulcherrima* (64%), *Samanea saman* (62%) and *Axonopus compressus* (61%) and the minimum root

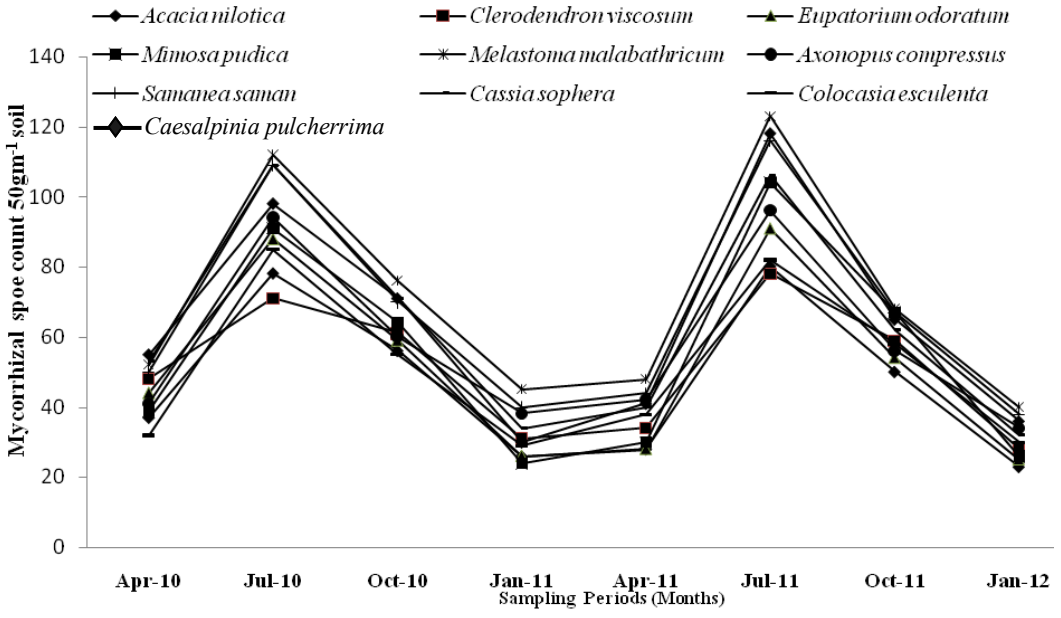


Fig 2: Monthly variation in mycorrhizal spore population 50gm^{-1}soil of different plant species growing in the non-polluted site.

colonization was estimated in *Eupatorium odoratum* (54%) and *Mimosa pudica* (52%) (Figures- 3 and 4).

Inter relationship of mycorrhizal association with soil Physio-chemical factors

The different soil parameters like N, P, K, Organic C (%), Ca and Mg were estimated in the polluted and non-polluted site. The polluted soil was less moist than the non-polluted one. The rhizosphere soil from polluted site was more alkaline than the non-polluted one. Likewise more temperature was recorded in the polluted site and less temperature was recorded in the non-polluted site. All physical parameters were recorded maximum in the month of July that gradually decreased from October till April except soil pH (Table- 1).

The soil samples from polluted and non-polluted site showed marked monthly variation in their chemical properties. Nitrogen, phosphorous and organic carbon (%) content of the rhizosphere soil gradually decreased from July to January and slightly increased in April. A similar trend of monthly variation was also observed in the non-polluted site as well. The soil phosphorus content of polluted site was found less than the non-polluted site. The soil calcium and magnesium content were also found more in the polluted site than the non-polluted site. The various trace metals like Cu, Ni and Zn were also estimated and found gradually decreased from July to January and then slightly increased from the month of April (Tables- 2 and 3).

Liner regression analyses were calculated to find out the influence of various edaphic factors on mycorrhizal colonization and mycorrhizal spore population. The results of regression analysis showed a positive and significant correlation coefficient(R) values between mycorrhizal spore population with soil moisture content ($r = 0.95$; $P < 0.01$; Fig. 5(a)), soil temperature ($r = 0.86$; $P < 0.01$; Fig. 5(b)), Nitrogen ($r = 0.81$; $P < 0.01$;Fig. 5(d)), Organic carbon ($r = 0.82$; $P < 0.01$; Fig. 5(g)), Calcium ($r = 0.84$; $P < 0.01$; Fig. 5(h)), Zinc ($r = 0.59$; $P < 0.01$; Fig. 5(k)), Cu ($r = 0.97$;$P < 0.01$; Fig. 5(i)) and Ni ($r = 0.92$; $P < 0.01$; Fig. 5(j)). The correlation coefficient with soil pH ($r = 0.75$; $P < 0.01$; Fig 5(c)) and soil phosphorus ($r = 0.75$; $P < 0.01$; Fig. 5 (e)) were however, negative and significant.

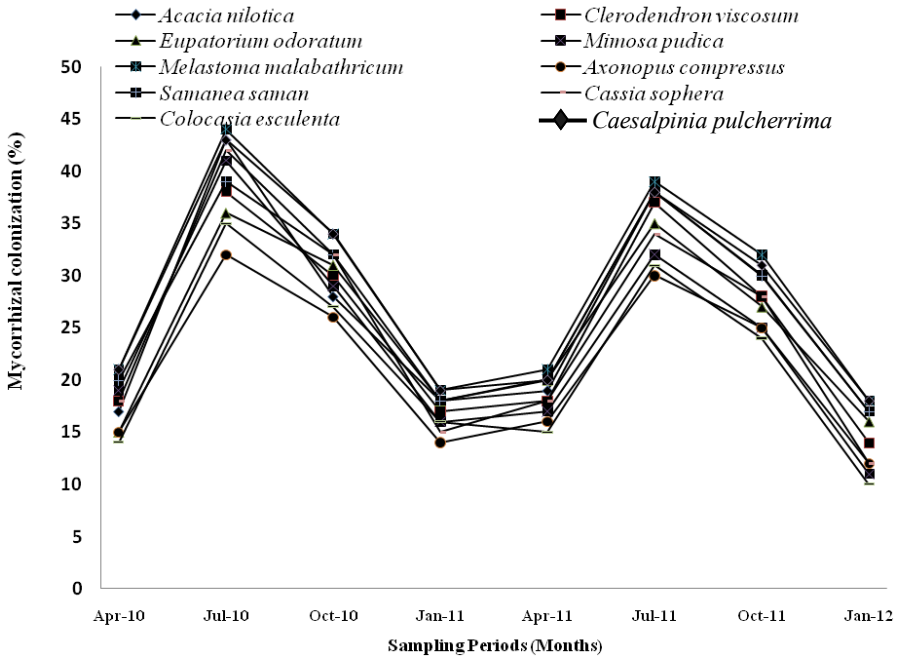


Fig 3: Monthly variation in mycorrhizal colonization (%) of different plant species growing in the polluted site.

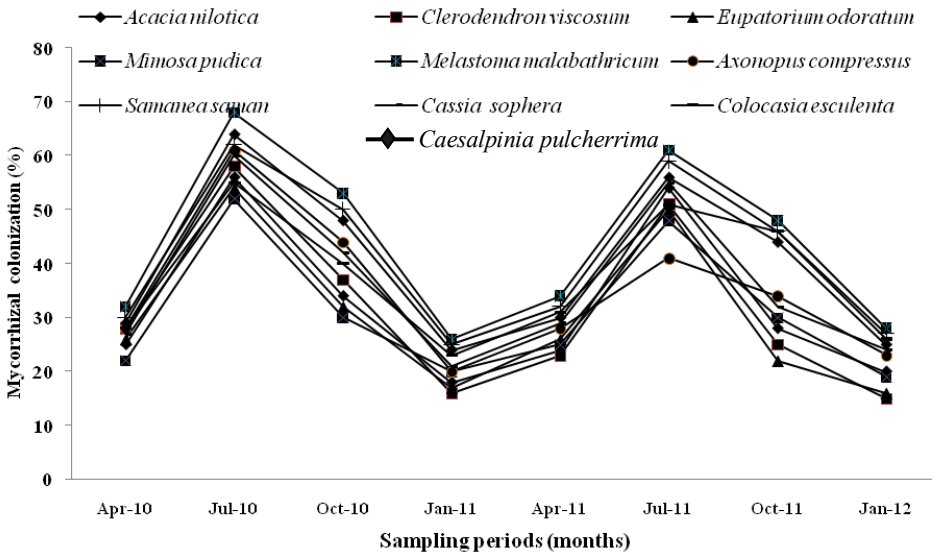


Fig 4: Monthly variation in mycorrhizal colonization (%) of different plant species growing in the non-polluted site.

The positive and significant correlation coefficient values were between mycorrhizal colonization and soil moisture content ($r = 0.86$; $P < 0.01$; Fig. 7(a)), soil temperature ($r = 0.70$; $P < 0.01$; Fig. 7(b)), Nitrogen ($r = 0.85$;$P < 0.01$; Fig. 7(d)), phosphorus ($r = 0.90$;$P < 0.01$; Fig. 7(e)), soil organic carbon ($r = 0.64$; $P < 0.01$; Fig. 7(f)), Calcium ($r = 0.97$;$P < 0.01$; Fig. 7(g)), copper ($r = 0.78$; $P < 0.01$; Fig. 7(i)) and Nickel ($r = 0.82$; $P < 0.01$; Fig. 7(j)) and Zinc ($r = 0.39$; $P < 0.01$; Fig. 7(k)) in the polluted site. The correlation coefficient with soil Mg and soil pH was however found negative and significant.

In the non-polluted site, a significant correlation coefficient values were estimated between mycorrhizal spore population soil pH ($r = 0.67$; $P<0.01$; Fig. 6(b)), soil moisture content ($r = 0.82$;$P < 0.01$; Fig. 6(a)), soil organic carbon ($r = 0.82$; $P < 0.01$; Fig. 6(f)), soil nitrogen ($r = 0.94$; $P<0.01$; Fig. 6(d)), soil phosphorus

Table 1: Monthly Variation in the physical properties of polluted & non-polluted soils.

Sampling Period	Physical parameters		
Months	**Moisture Content (%)**	**pH**	**Soil Temperature (C^0)**
April,10	7.8 ± 0.08 (16.3 ± 0.05)	6.9 ± 0.08 (4.10 ± 0.05)	23.1 ± 0.08 (15.2 ± 0.03)
July,10	14.4 ± 0.12 (24.8 ± 0.05)	6.1 ± 0.05 (4.80 ± 0.06)	27.5 ± 0.03 (21.5 ± 0.05)
October,10	11.3 ± 0.05 (18.8 ± 0.03)	6.7 ± 0.03 (4.30 ± 0.03)	22.8 ± 0.03 (17.8 ± 0.08)
January,11	5.7 ± 0.03 (8.2 ± 0.08)	7.1 ± 0.03 (4.48 ± 0.13)	19.8 ± 0.06 (14.6 ± 0.03)
April,11	8.1 ± 0.03 (14.2 ± 0.06)	6.9 ± 0.05 (4.00 ± 0.05)	22.8 ± 0.03 (15.4 ± 0.08)
July,11	16.5 ± 0.05 (23.8 ± 0.05)	6.5 ± 0.03 (5.30 ± 0.03)	28.2 ± 0.06 (21.0 ± 0.03)
October,11	12.5 ± 0.03 (18.2 ± 0.03)	6.9 ± 0.03 (4.60 ± 0.03)	23.0 ± 0.05 (18.2 ± 0.08)
January,12	6.2 ± 0.03 (8.4 ± 0.05)	7.2 ± 0.03 (4.40 ± 0.05)	18.7 ± 0.06 (15.1 ± 0.05)

Data are represented in mean ±SE; Value in parentheses represents the data from non-polluted site

Table 2: Monthly Variation in the chemical properties of polluted and non-polluted soil.

Sampling periods Months	Chemical parameters								
	N (mg/g)	P (mg/g)	K (mg/g)	Organic C%	Mg (mg/g)	Ca (mg/g)	Cu (ppm)	Ni (ppm)	Zn (ppm)
April,10	0.3125±0.080 (0.0217±0.050)	0.0057±0.06 (0.0027±0.03)	0.21±0.02 (0.050±0.03)	1.78±0.08 (0.413±0.03)	3.24±0.05 (0.132±0.03)	4.76±0.03 (0.12±0.05)	0.034±0.02 BDL	0.013± 0.05 BDL	0.317±0.04 BDL
July,10	0.4270±0.060 (0.0740±0.030)	0.0016±0.05 (0.0062±0.06)	0.38±0.05 (0.046±0.06)	2.17±0.06 (0.615±0.05)	1.89±0.06 (0.081±0.08)	5.79±0.06 (0.07±0.03)	0.075±0.05 BDL	0.034±0.03 BDL	0.358±0.06 BDL
October,10	0.4100±0.050 (0.0380±0.030)	0.0035±0.07 (0.0047±0.03)	0.26±0.05 (0.032±0.03)	1.86±0.07 (0.578±0.03)	2.24±0.07 (0.118±0.05)	5.31±0.02 (0.068±0.06)	0.047±0.07 BDL	0.022±0.06 BDL	0.297±0.05 BDL
January,11	0.3630±0.060 (0.0240±0.050)	0.0047±0.05 (0.0020±0.03)	0.18±0.06 (0.017±0.05)	1.23±0.05 (0.439±0.06)	2.08±0.03 (0.127±0.06)	4.86±0.08 (0.079±0.07)	0.023±0.08 BDL	0.008±0.02 BDL	0.278±0.03 BDL
April,11	0.3290±0.070 (0.0260±0.030)	0.0062±0.06 (0.0034±0.05)	0.31±0.07 (0.080±0.04)	1.82±0.07 (0.424±0.03)	3.19±0.07 (0.141±0.05)	4.67±0.05 (0.17±0.03)	0.041±0.06 BDL	0.016±0.03 BDL	0.324±0.04 BDL
July,11	0.4510±0.050 (0.0870±0.030)	0.0021±0.03 (0.0071±0.05)	0.46±0.05 (0.057±0.03)	2.34±0.07 (0.648±0.03)	1.75±0.57 (0.105±0.38)	5.63±0.05 (0.11±0.06)	0.087±0.03 BDL	0.041±0.05 BDL	0.349±0.03 BDL
October,11	0.3800±0.057 (0.0420±0.060)	0.0031±0.06 (0.0039±0.03)	0.37±0.05 (0.037±0.06)	1.89±0.06 (0.580±0.05)	2.15±0.03 (0.120±0.04)	5.37±0.03 (0.07±0.05)	0.052±0.06 BDL	0.029±0.06 BDL	0.285±0.06 BDL
January,12	0.3200±0.030 (0.0280±0.028)	.0049±0.07 (0.0051±0.03)	0.22±0.03 (0.022±0.06)	1.28±0.03 (0.447±0.02)	2.01±0.05 (0.129±0.06)	4.77±0.06 (0.082±0.02)	0.029±0.05 BDL	0.006±0.07 BDL	0.275±0.04 BDL

Data are represented in mean ±SE; BDL=Below Detectable Limit; Value in parentheses represents the data from non-polluted site

Table 3: Monthly Variation in the Mycorrhizal spore population and Mycorrhizal root colonization (%) in 50gm^{-1} soil of polluted and non-polluted sites

Sampling Periods Months	Endogonaceous Spore Population(50gm^{-1})	Mycorrhizal colonization (%)
April,10	24 ± 0.6 (52 ±0.8)	21 ± 0.8 (32 ± 0.6)
July,10	54 ± 0.5 (118 ±0.8)	44 ± 0.3 (68 ± 0.4)
October,10	39 ± 0.3 (75 ±0.8)	34 ± 0.5 (53 ± 0.3)
January,11	18 ± 0.5 (46 ±0.5)	21 ± 0.5 (26 ± 0.3)
April,11	26 ± 0.5 (49 ±0.8)	19 ± 0.5 (34 ± 0.6)
July,11	61 ± 0.5 (124 ±0.5)	39 ± 0.3 (61 ± 0.5)
October,11	35 ± 0.5 (68 ±0.8)	32 ± 0.3 (48 ± 0.8)
January,12	20 ± 0.5 (40 ±0.5)	18 ± 0.3 (28 ± 0.4)

Data are represented in mean ±SEM; Value in parentheses represents the data from non-polluted site

(r = 0.85; P < 0.01; Fig. 6(e)) and soil magnesium (r = 0.77; P < 0.01; Fig. 6(g)).

In the non-polluted site, the mycorrhizal colonization was found significantly and positively correlated with soil moisture content (r = 0.80; P < 0.01; Fig. 8(a)), soil temperature (r = 0.94; P < 0.01; Fig. 8(c)), soil pH (r = 0.54; P < 0.01; Fig. 8(b)) soil Nitrogen (r = 0.79; P < 0.01; Fig. 8(d)), phosphorus (r = 0.92; P < 0.01; Fig. 8(e)), soil organic carbon (r = 0.90; P < 0.01; Fig. 8 (f)), Magnesium (r = 0.85; P < 0.01; Fig. 8(h)). The correlation coefficient with soil Calcium was however found negative and significant.

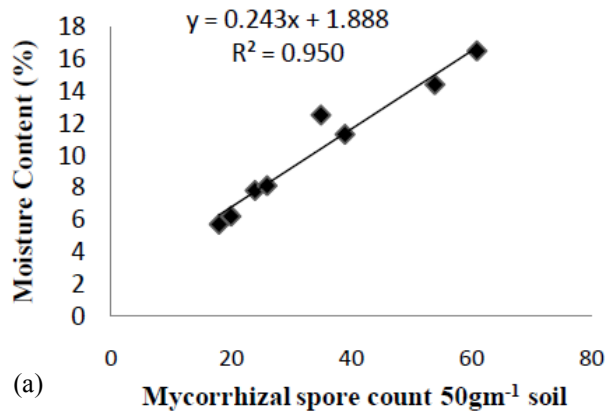
The present experimental findings revealed the relationship of mycorrhizal spore population and mycorrhizal colonization with various physio-chemical properties of soil polluted with trace metals. The low intensity of root colonization and low spore count in the polluted site may be attributed to the sensitivity of endomycorrhizal fungi to various soil pollutants. This may be due to the alkaline pH, higher soil temperature due to the deposition of more amounts of Calcium and trace metals that might have adversely affected the sporulation and colonization ability of the mycorrhizal fungi as reported by Schenck and Smith (1982). Rohyadi *et al.,* (2004) also observed that the relative growth improvement by mycorrhizas was highest at pH 4.7 and the same decreased as the pH increased. The presence of trace metals in the polluted soil may be responsible for less percentage of root colonization in the polluted site. AM spore population decreased with increased amount of trace metals in the soil (Val *et al*., 1999; Hayes *et al*., 2003).The negative correlation with soil Phosphorous, Magnesium and pH is may be responsible for the less percentage of root colonization in the plants. High alkalinity in the soil was also responsible for decrease in the number of spores as well as root colonization in the polluted soil. The spore population and mycorrhizal root colonization of AMF fungi were found decreased by the higher levels of heavy metals in the soil. Our results also supports the findings of (Shah *et al.,* (2010); Biro *et al.,* (2005); Göhre and Paszkowski (2006); Mathur *et al.,* (2007)).

Among the isolated genera of AM fungi, *Glomus* was the most dominant AM genus isolated during the present investigation followed by *Gigaspora* and *Scutellospora* sp. Dominance of *Glomus* sp in the polluted soil may be due to its higher metal tolerance capacity as reported earlier by various workers (Martina and Vosatka 2005; Carrasco *et al.,* 2011; Chen *et al.,* 2007; Zaefarian *et al.,* 2010). The decline of AM fungal occurance (propagule density) and infectivity in trace metal polluted site which can be used as bioindicators of

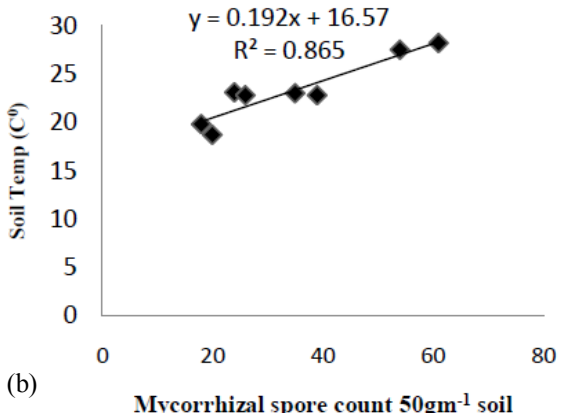
soil contamination (Citterio *et al.*, 2005; Liao *et al.*, 2003).

CONCLUSION:

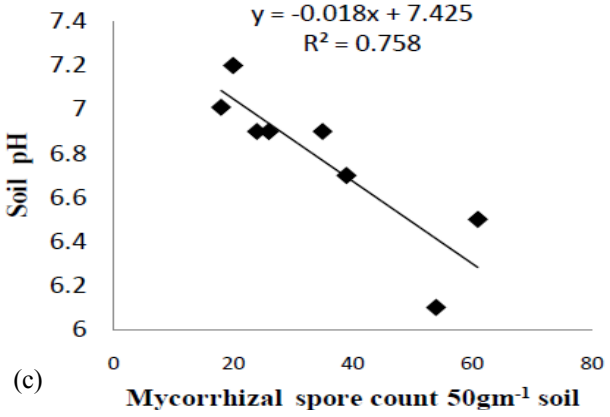
Our study suggests that the effluents and the solid wastes dumped by the paper mill have high concentration of trace metals that changed the other physical and chemical properties of the soil. The indigenous AM isolates existing naturally which are isolated from trace metal polluted soils are reported efficiently to colonize plant roots in trace metal-stressed environments by significantly correlated with various physic-chemical properties of the soil. It is therefore of great importance that we combine selected plants with specific AM fungal isolates adapted to high concentrations of trace metal in future research for phytoremediation programes. Thus, the isolated strains of AM fungi can be of great interest since they can be used for inoculation of the plant species and the present study provides evidences for the potential use of the



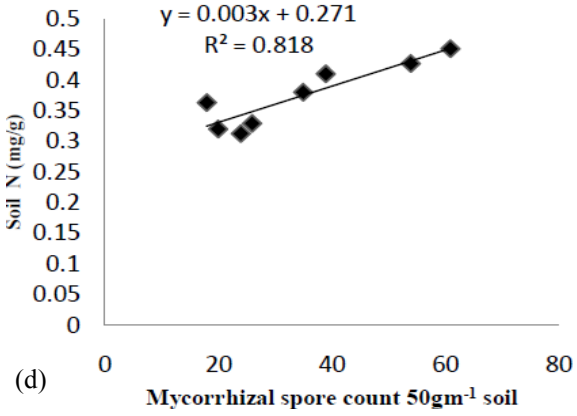
(a)



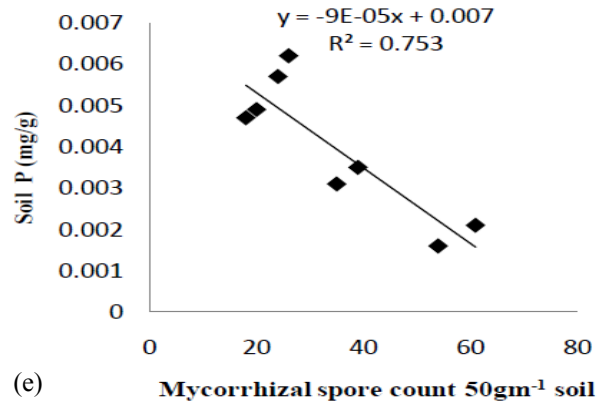
(b)



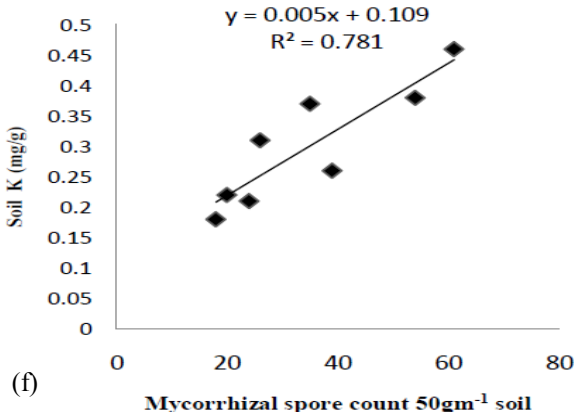
(c)



(d)



(e)



(f)

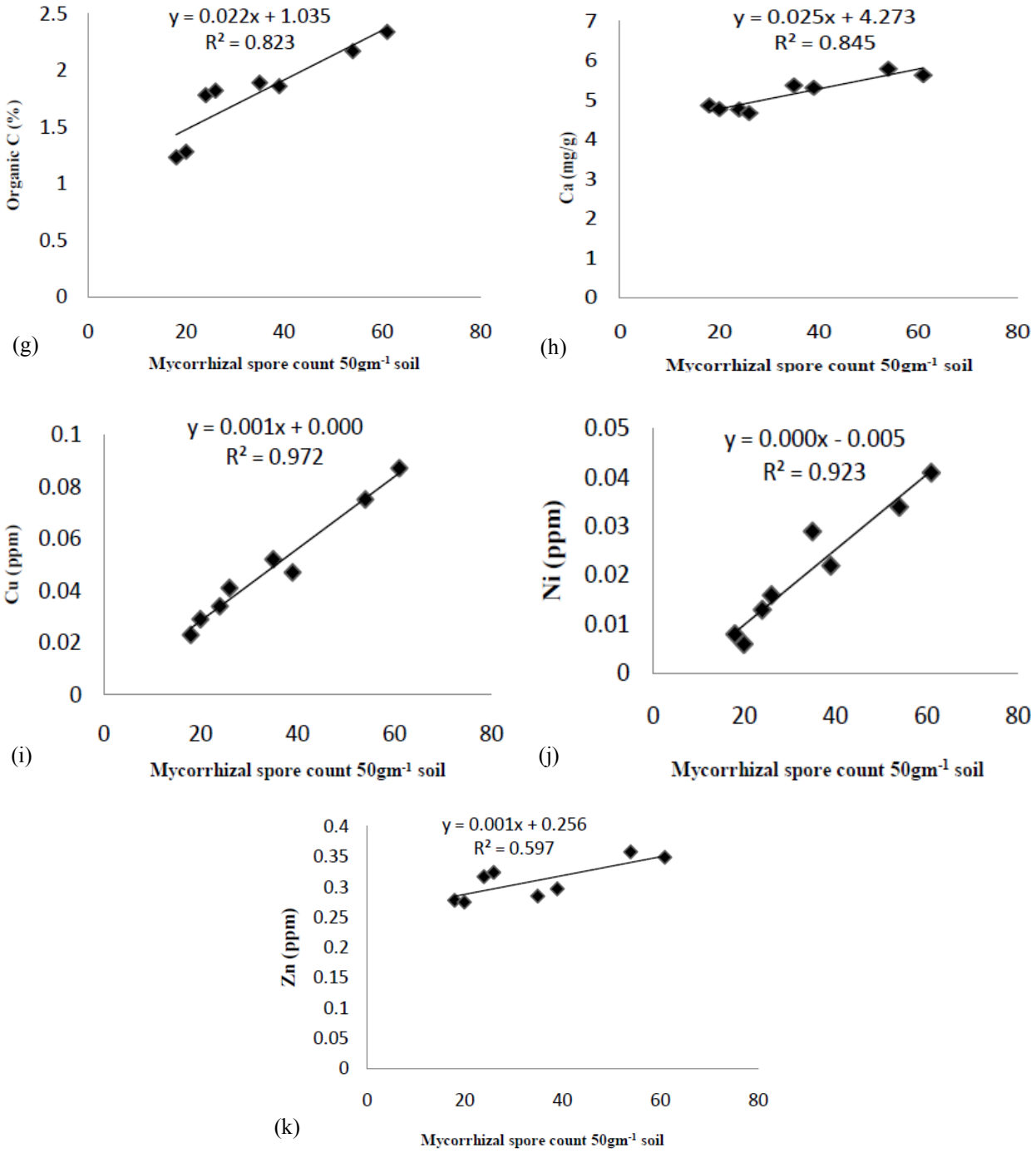


Figure 5: Mycorrhizal spore population 50gm^{-1} soil (X) expressed as a function of soil physio-chemical factors (Y) in the polluted site.Regression is drawn only for statistically significant relationship ($p < 0.01$). (MC=Moisture Content; Soil temp(C^0),soil pH,Nitrogen (N), Potassium (K), Phosphorus (K),Organic Carbon (%),Calcium (Ca),Copper (Cu), Nickel (Ni) and Zinc (Zn)).

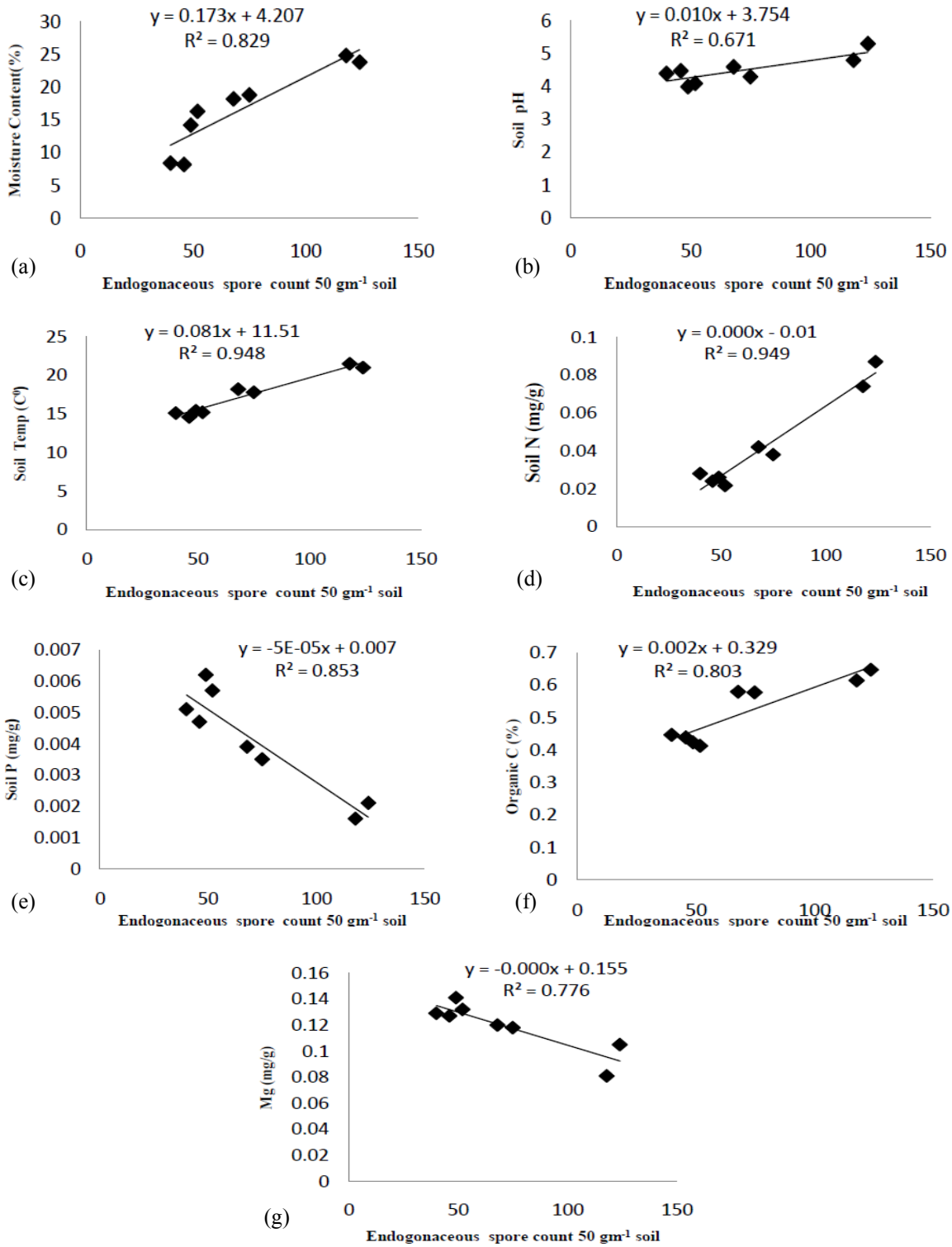
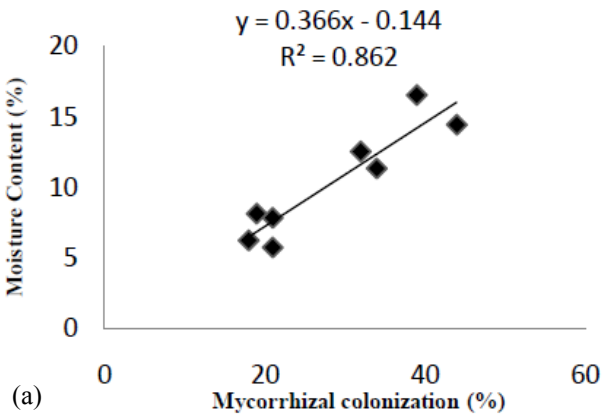
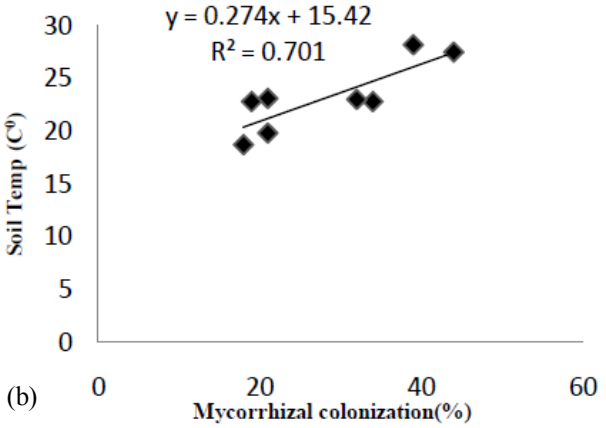


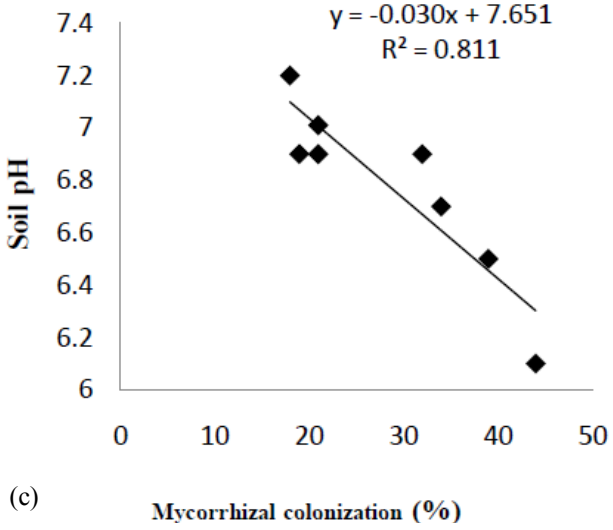
Figure 6: Mycorrhizal spore population 50gm^{-1} soil (X) expressed as a function of soil physio-chemical factors (Y) in the non-polluted site.Regression is drawn only for statistically significant relationship ($p < 0.01$). (MC=Moisture Content; Soil temp(C^0),Soil pH, Nitrogen(N), Potassium(K),Phosphorus(P),Organic Carbon (%),Magnesium(Mg)).


y = 0.366x - 0.144
R² = 0.862
Moisture Content (%)
Mycorrhizal colonization (%)

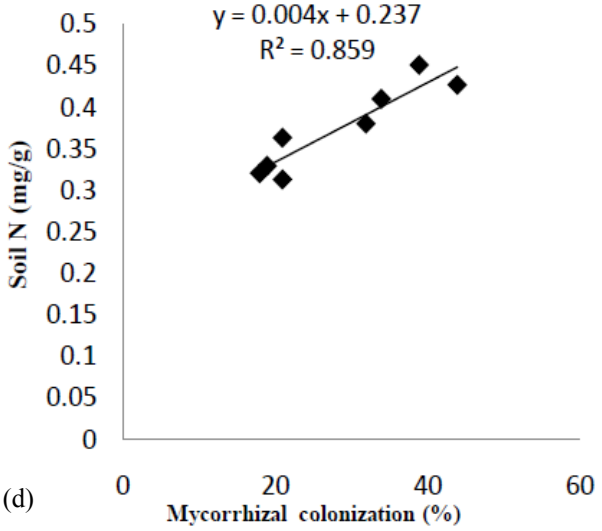
(a)


y = 0.274x + 15.42
R² = 0.701
Soil Temp (C°)
Mycorrhizal colonization(%)

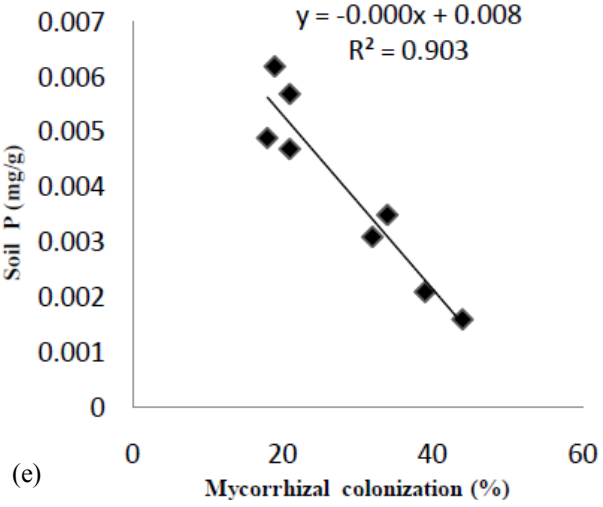
(b)


y = -0.030x + 7.651
R² = 0.811
Soil pH
Mycorrhizal colonization (%)

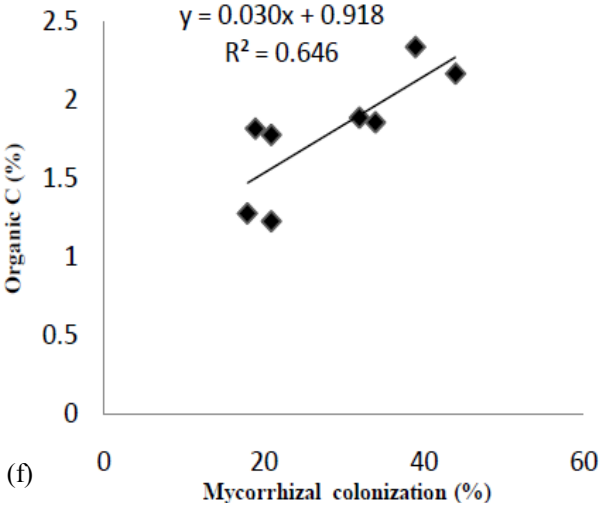
(c)


y = 0.004x + 0.237
R² = 0.859
Soil N (mg/g)
Mycorrhizal colonization (%)

(d)


y = -0.000x + 0.008
R² = 0.903
Soil P (mg/g)
Mycorrhizal colonization (%)

(e)


y = 0.030x + 0.918
R² = 0.646
Organic C (%)
Mycorrhizal colonization (%)

(f)

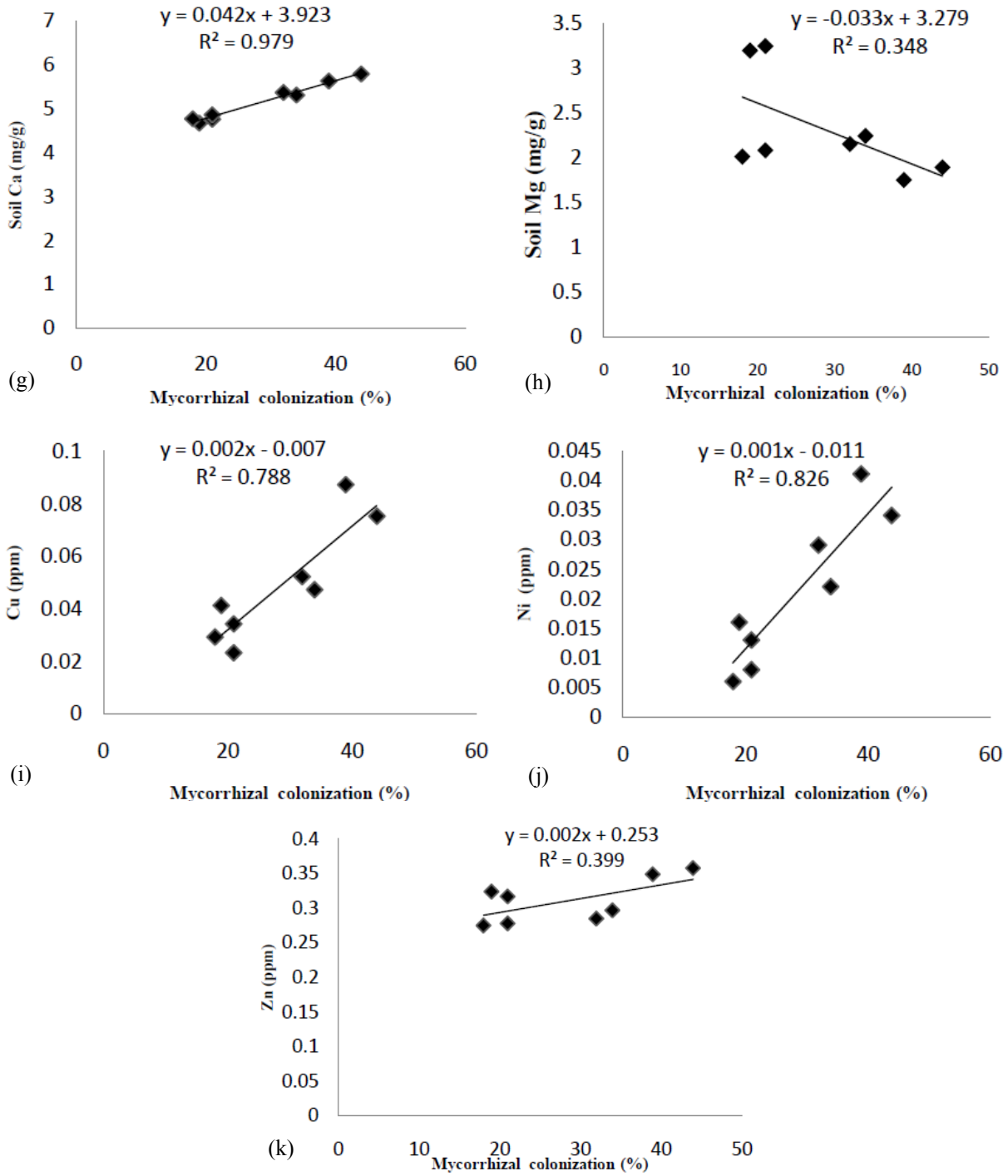
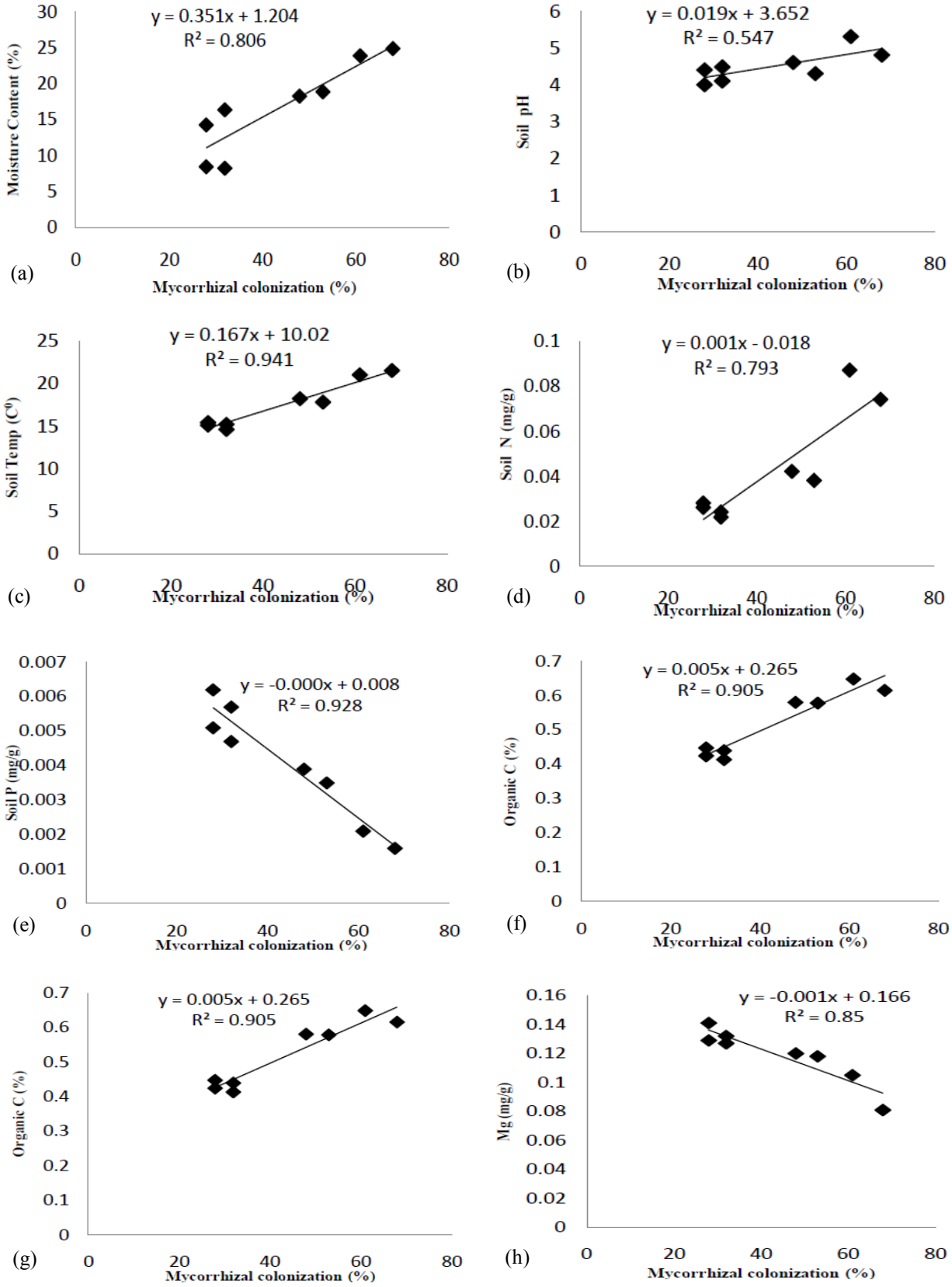
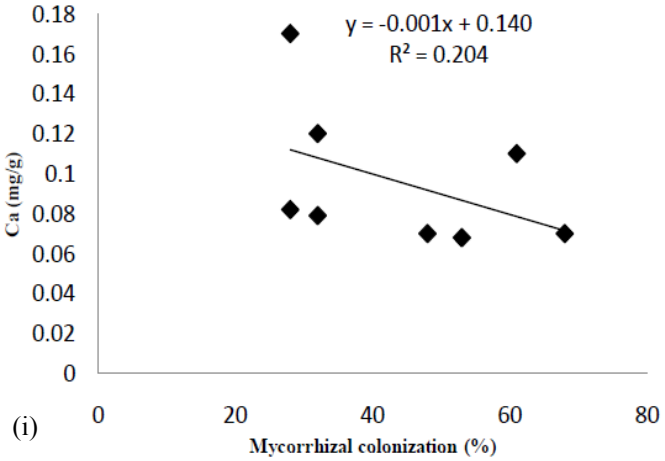


Figure 7: Mycorrhizal colonization (X) expressed as a function of soil physio-chemical factors (Y) in the polluted site.Regression is drawn only for statistically significant relationship ($p < 0.01$). MC=Moisture Content; Soil temp(C^0),Nitrogen (N), Phosphorous (P),Organic Carbon (%),Calcium (Ca),Magnesium (Mg),Copper (Cu),Nickel (Ni) and Zinc (Zn)).


y = 0.351x + 1.204
R² = 0.806
Moisture Content (%)
Mycorrhizal colonization (%)
(a)
y = 0.019x + 3.652
R² = 0.547
Soil pH
Mycorrhizal colonization (%)
(b)
y = 0.167x + 10.02
R² = 0.941
Soil Temp (C⁰)
Mycorrhizal colonization (%)
(c)
y = 0.001x - 0.018
R² = 0.793
Soil N (mg/g)
Mycorrhizal colonization (%)
(d)
y = -0.000x + 0.008
R² = 0.928
Soil P (mg/g)
Mycorrhizal colonization (%)
(e)
y = 0.005x + 0.265
R² = 0.905
Organic C (%)
Mycorrhizal colonization (%)
(f)
y = 0.005x + 0.265
R² = 0.905
Organic C (%)
Mycorrhizal colonization (%)
(g)
y = -0.001x + 0.166
R² = 0.85
Mg (mg/g)
Mycorrhizal colonization (%)
(h)



(i)

Figure 8: Mycorrhizal colonization (X) expressed as a function of soil physio-chemical factors (Y) in the non-polluted site.Regression is drawn only for statistically significant relationship ($p < 0.01$). (MC = Moisture Content; Soil temp(C^0),Soil pH, Nitrogen(N), Potassium (K),Phosphorus (P),Organic Carbon (%),Magnesium (Mg) and Calcium (Ca)).

plant species in combination with AM fungi in the paper mill polluted with paper mill effluents contaminated with various trace metals.

ACKNOWLEDGEMENT:

The authors are grateful to the Department of Life Science, Microbiology Laboratory, Assam University (Silchar), India for providing the laboratory facilities.

REFERENCES:

Almas AR, Bakken LR and Jan Mulder. 2004. Changes in tolerance of soil microbial communities in Zn and Cd contaminated soils. Soil Biol Biochem. 36(5): 805–813.

Andrew B, Brown MV, Steven DS and Peter HT. 2013. Microbial community responses to anthropogenically induced environmental change: towards a systems approach. Ecol Lett. 16 (Supplement S1): 128-139.

Barea JM and Jeffries P. 1995. Arbuscular mycorrhizas in sustainable soil plant systems. In: B. Hock and A. Varma (eds) Mycorrhiza, structure, Function, Molecular Biology and biotechnology. Springer-Verlag, Heidelberg. 521-559.

Biró B, Posta K, Füzy A, Kádár I and Németh T. 2005. Mycorrhizal Functioning as part of the Survival Mechanisms of Barley (*Hordeum vulgare* L.) at Long-term Heavy Metal Stress, Acta Biol.Szegedien. 49 (1-2): 65-67.

Carrasco L, Azcon R, Kohler J, Roldán A and Caravaca F. 2011. Comparative effects of native filamentous and arbuscular mycorrhizal fungi in the establishment of an autochthonous, leguminous shrub growing in a metal-contaminated soil. Sci Total Environ., 409(6): 1205-1209.

Chen BD, Zhu Y.-G, Duan J, Xiao XY and Smith SE. 2007. Effects of the arbuscular mycorrhizal fungus *Glomus mosseae* on growth and metal uptake by four plant species in copper mine tailings. Environ Pollut. 147(2): 374-380.

Citterio S, Prato N, Fumagalli P. Aina R, Massa N, Santagostino A, Sgorbati S and Berta G. 2005. The arbuscular mycorrhizal fungus Glomus mosseae induces growth and metal accumulation changes in Cannabis

sativa L. Chemosphere. 59(1): 21-29.

Glassman SI and Casper BB. 2012. Biotic contexts alter metal sequestration and AMF effects on plant growth in soils polluted with heavy metals. Ecology. 93(7): 1550-1559.

Gerdemann JW and Nicolson TH. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc., 46(2): 235-244.

Göhre V and Paszkowski U. 2006. Contribution of the Arbuscular Mycorrhizal Symbiosis to Heavy Metal Phytoremediation. Planta. 223(6): 1115-1122.

Gomez KA and Gomez AA. 1984. Statistical Procedures for Agricultural Research (2nd edn), An International Rice Research Institute book, A Wiley-Interscience Publication, John Willey and Sons, New York.

Hayes WJ, Chaudhry TM, Buckney RT and Khan AG. 2003. Phytoaccumulation of Trace Metals at the Sunny Corner Mine, New South Wales with Suggestions for a Possible Remediation Strategy, Aust J Toxicol., 9(1):69-82.

Jackson ML. 1985. Soil chemical analysis, 2nd edition, Madison, WI, USA.

Joner EJ and Leyval C. 2003. Phytoremediation of organic pollutants using mycorrhizal plants: a new aspect of rhizosphere interactions. Agronomie. 23(5-6): 495-502.

Khan AG, Kuek C, Chaudhry TM, Khoo CS and Hayes WJ. 2000. Role of plants,mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere. 41(1-2):197-207.

Leyval C, Turnau K and Haselwandter K. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza. 7(3): 139-153.

Liao JP, Lin XG, Cao ZH, Shi YQ and Wong MH. 2003. Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. Chemosphere. 50(6): 847-853.

Mathur N, Bohra JSS, Quaizi A and Vyas A. **2007**. Arbuscular Mycorrhizal Fungi: A Potential Tool for Phytoremediation, J Plant Sci., 2(2): 127-140.

Martina J and Vosatka M. 2005. Response to Cadmium of *Daucus carota* hairy roots dual cultures with *Glomus intraradices* or Gigaspora margarita. Mycorrhiza. 15(3): 217-224.

Olexa TJ, Gentry TJ, Hartel PG. Wolfb DC, Fuhrmannc JJ and Reynoldsd CM. 2000. Mycorrhizal Colonization and microbial community structure in the rhizosphere of annual ryegrass grown in pyrene-amended soils. Int J Phytol., 2(3): 213-231.

Phillips JM and Hayman DS. 1970. Improved procedures for cleaning and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc., 55(1): 158-161.

Rahmanian M, Khodaverdiloo H, Rezaee DY and Rasouli SMH. 2011. Effects of Heavy Metal Resistant Soil Microbes Inoculation and Soil Cd Concentration on Growth and Metal Uptake of Millet, Couch Grass and Alfalfa. Afr J Microbiol Res., 5(4): 403-410.

Rohyadi A, Smith FA, Murray RS and Smith SE. 2004. Effects of pH on mycorrhizal colonisation and nutrient uptake in cowpea under conditions that minimise confounding effects of elevated available aluminium. Plant Soil. 260(1-2): 283-290.

Shah FR, Ahmad N, Masood KR. Peralta-Videa JR and Ahmad FuD. 2010. Heavy Metal Toxicity in Plants.

In: Ashraf, M. Ozturk M. and S.A. Ahmad (eds) Plant Adaptation and Phytoremediation, Springer Dordrecht Heidelberg London, New York.71-98.

Schenck NC and Smith GS. 1982. Responses of six species of vesicular arbuscular mycorrhizal fungi and their effects on soybean at four-soil temperatures. New Phytol., 92(2):193-201.

Schenck, NC and Perez Y. 1990. Manual for the Identification of VA Mycorrhizal Fungi. (3rd edn). Gainesville, Florida, Synergistic Publications.

Smith SE and Read DJ. 2008. Mycorrhizal Symbiosis Academic press London.

Val CD, Barea, JM and Azcon-Aguilar C. 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy- metal- contaminated soils. Appl Environ. Microbiol., 65(2): 718-723.

Zaefarian F, Rezvani M, Rejali F, Ardakani MR and Noormohammadi G. 2010 Ability of *Glomus mosseae*-Alfalfa (*Medicago sativa* L.) Association for Heavy Metal Phytoextraction from Soil. Environ Sci., 7(3): 77-90.

Zarei M, Hempel S, Wubet T, Schäfer SH, Savaghebi G, Jouzani GS, Nekouei MK and Buscot F. 2010. Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. Environ. Pollu., 158(8): 2757-2765.