

EFFECT OF MYCORRHIZAL INOCULATION ON THE GROWTH AND PHYTOEXTRACTION OF HEAVY METALS BY MAIZE GROWN IN OIL CONTAMINATED SOIL

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Abstract

Pot experiments were conducted to investigate the effect of AM (*Glomus mosseae*) fungi inoculation (M) on the growth of maize and phytoextraction of selected heavy metals from a soil contaminated with crude oil (C). Four soil treatments, each with three replicates i.e., C⁺M⁺, M⁺, C⁺ and control (without oil and inoculum) were conducted. Half of the pots with the soil treatments were planted with singly sown (SS) and the other half with densely sown i.e., four maize seedlings (DS). Various plant growth attributes were measured at weekly intervals Cu²⁺, Ni²⁺, Pb²⁺ and Cd²⁺ in the soil, root and shoot of maize plants were determined separately. Inoculation by AM promoted the vegetative growth attributes in both treatments viz., C⁺M⁺ and M⁺. AM inoculation also promoted the hyperextraction of heavy metals from C⁺M⁺ soils, but inhibited by soils treated with M⁺. High planting density i.e., DS also promoted phytoextraction of heavy metals from uncontaminated (M⁺) soils, but had minimal effect on phytoextraction from oil contaminated soils (C⁺). Planting density complemented the promotive effect of AM inoculation on phytoextraction of heavy metals from C⁺ soils. The hyperextraction of selected metals from soil is more favored by planting density in C⁺ soils, whereas AM inoculation tends to exclude heavy metals from potted plants. However, in case of C⁺M⁺ soils, AM inoculation promotes the hyperextraction of metals more than planting density. While the combination of the two phenomena act synergistically to promote metal hyperextraction from C⁺M⁺ as well as M⁺ soils.

Introduction

Maize [*Zea mays* (L.)] is the world's third most important cereal grain, which is primarily grown for grains and fodder. In Nigeria, maize cultivation is gaining popularity. Its production during the period rose from 7.1 million tons in 2006 to 7.8 million tons in 2007. This figure could be doubled if the recommendations of the researchers are implemented (Anon., 2009; Ahmad *et al.*, 2010). Phytoremediation, also called green remediation, botano-remediation, agromediation, or vegetative remediation is considered a publicly appealing (green) remediation technology that uses vegetation and associated microbiota, soil amendments and agronomic techniques to remove, contain, or render the heavy metals harmless in the soil (Vysloužilová *et al.*, 2003; Helmissaari *et al.*, 2007). Heavy metal contamination of soils is a major environmental problem worldwide and phytoextraction or phytoremediation has emerged as a potential cost-effective and environmentally sustainable technique for removing toxic metals from soils (McGrath & Zhao, 2003; Tanvir & Siddiqui, 2010). Plants have a natural propensity to take up metals from polluted soil (Lasat, 2000).

On account of anthropogenic activities our natural habitats are endangered. The restoration of such degraded habitats using sustainable, low input cropping systems with the aim of maximizing yield of crop plants is the need of the hour. Thus incorporation of the natural roles of the beneficial micro-organisms in maintaining soil fertility and plant productivity is gaining much more attention. Research revealed that AM fungi assist the host plant in P and N uptake and also some of the relatively immobile micronutrients and trace elements viz., Cu²⁺, Fe²⁺, Zn²⁺, Ni²⁺, Pb²⁺ and Cd²⁺ (Garg & Chandel, 2010). Studies also revealed that maize can survive soil contamination of about 21% crude oil and still produce fresh cob yield of about 60% than over normal soil (Ayotamuno & Kogbara, 2007). There was

reduction in the toxicity of the soils treated with *Pleurotus tuber-regium* (Fr). The continued growth of the plant in the soils contaminated with diesel fuel @ 2.50 and 5.00% showed no significant difference between them and the control treatment using leaf area, plant height, dry weight and root length indices (Ogbo *et al.*, 2010). They also stated that the fungus was able to reduce the toxicity of diesel fuel contaminated substrates when compared with their respective control in which no remediation occurred. Based upon plant biomass production in contaminated soil, maize and soybean seedlings showed the greatest potential to enhance remediation (Issoufi *et al.*, 2006). Researchers further pointed that plant height and shoot biomass are good indicators of plant health and the sustenance of plant growth by the treated substrates or soil is an indication of enhanced bioremediation (Banks *et al.*, 2003).

Crude oil is a mixture of aliphatic, aromatic, heterocyclic, asphaltene/tar hydrocarbons, ranging in size from C₆ to > C₅₀. These hydrocarbons can be degraded by various processes, including photo-oxidation, microbial action and natural rhizosphere action (Greenberg *et al.*, 2007). The contamination of soils by petroleum hydrocarbons causes drastic changes in microbiological, chemical and physical properties of soil. The soil by design or accident has been the recipient of myriads of waste products and chemicals used in modern society (Brady & Weil, 1998). Large quantities of locally grown food that are planted in areas contaminated by toxic trace elements may be consumed by the residents, exposing the population to trace element poisoning (Liasu *et al.*, 2006).

Research revealed that AM fungi promote increased yield in crops due to increased nutrient uptake especially in marginal soils (Liasu *et al.*, 2002). This root fungus facilitates resistance to soil borne pathogens and also promotes resistance to soil pollutants including heavy metals and hydrocarbons in some cases (Killham & Firestone, 1983). Researchers also reported that AM fungi

promotes the uptake of metal ions (Tonin *et al.*, 2001; Liao *et al.*, 2003; Whitefield *et al.*, 2004; Citterio *et al.*, 2005), or decreased uptake (Weissemhorn *et al.*, 1995; Chen *et al.*, 2003) and or having no effects on metal uptake (Trotta *et al.*, 2006). While conflicting reports have been given on the effects of AM fungi on phytoextraction of metal including heavy metals from polluted soil (Lasat, 2002; Liu *et al.*, 2006; 2007). Over 400 taxa of plant hyperaccumulators of heavy metals have been identified, but most of them are low biomass producers and exotic species. However, Wuana & Okieimen (2010) stated that maize is a widely grown cereal with promising attributes of a heavy metal accumulator. The present study was mainly aimed to investigate the potential use of this robust tropical inoculated crop on the growth and phytoextraction of heavy metals from soil polluted with crude oil.

Materials and Methods

Pre-planting operation: The seeds of improved variety (DMRV-W) of maize (*Zea mays* L.) were obtained from Oyo State Agricultural Development Programme (OYSADEP) Centre, Ogbomoso, Oyo State, Nigeria. Seedlings boxes (70 x 40cm) were raised in sterilized saw-dust bed in sterile boxes.

Nursery preparation: The boxes were filled with saw-dust and watered prior to seed planting. The seeds were then evenly planted and maintained for one week prior to transplanting.

Soil collection: Good garden soil of sandy loam texture was collected from an area within the campus of Ladoke Akintola, University of Technology (LAUTECH), Ogbomoso and placed in clean, pierced polythene bags previously sterilized with 90% ethanol. These polythene bags were then filled with garden soil.

Crude oil samples: Crude oil samples were collected from oil exploration sites of Nigerian Agip Oil Company (NAOC) and classified as API light offshore crude according to the US department of petroleum classification.

AM inoculum: Arbuscular mycorrhizal (AM) fungal inoculum of *Glomus mosseae* consisted of soil containing spores, hyphal fragment, and infected fine roots of maize (i.e., trap plant) were thoroughly mixed together. The sample used was collected from the stock maintained in the soil/environmental biology unit of the Department of Pure and Applied Biology LAUTECH Ogbomoso.

Soil treatment: Soil contamination on the field was simulated in pots as follows: Planting pots (19cm height x 26cm diameter) were filled with 10kg good garden soil (sandy loam) to about three quarter of the depth of the pot and 50ml of crude oil added to the soil in each pot. The composting manure was added in the ratio of 1:1. The pots were then filled to the top with the same garden soil and left standing for about 15 days just to allow initiation of decomposition. Some pots containing uncontaminated soils were set aside as control. The process was replicated for soils inside pots that were destined for inoculation. Inoculation with *Glomus mosseae* was done by putting 50 g of inoculum in the

planting hole before planting maize seedlings in the pots designated for inoculation. There were 4 treatments in all and were tagged appropriately as follows:-

1. Soils contaminated with crude oil and inoculated with *Glomus mosseae* (C⁺M⁺)
2. Soils without crude oil contamination but inoculated with *Glomus mosseae* (M⁺)
3. Soils contaminated with crude oil without *Glomus mosseae* (C⁺) and
4. Soils without crude oil contamination and without *Glomus mosseae* (control)

Inoculum preparation: The arbuscular mycorrhizal fungus species employed was *Glomus mosseae*. The inoculum used in this experiment was prepared from the inoculum stock kept and maintained in the laboratory of Department of Pure and Applied Biology (LAUTECH), Ogbomoso. The inoculum used consisted of soil containing spores (800 to 1000/100g dry soil), hyphal fragments and fine roots of maize infected with *Glomus mosseae*.

Transplanting of maize seedlings: Twelve hours before transplanting all the planting bags were watered and the seedlings were transplanted following evening in order to give enough time to transplanted seedlings to become acclimatized with their new environment before sunrise and as such safeguarding them against transpiration shock. Maize seedlings were transplanted into already prepared pots (19cm height x 26cm diameter) i.e., contaminated and uncontaminated (control).

Experimental designs: In all there were four designates viz., C⁺M⁺, M⁺, C⁺ and control. Each of them were designated singly sown (SS) pot with only one maize seedling transplanted into it, and densely sown (DS) pot with four maize seedlings transplanted into it. Both the SS and DS pots were replicated thrice. Planting holes were dug inside the soil of each planting bag. The seedlings designated for inoculation were stood in the holes with the roots completely buried in the inoculums filled hole within the soil of the planting bags, while those of non-inoculated had their roots covered up with the soil initially scooped to create the planting holes. All the seedlings were watered twice daily for a period of nine weeks consecutively.

Data collection

Growth measurements: Measurement of plant growth parameters commenced by the first week after transplanting. Growth parameters such as plant-height (cm), stem-girth (cm), leaf-length (cm), and leaf-width (cm) were recorded regularly at weekly intervals. The leaves selected for measurement were of the fully expanded new leaves. The stem girth was measured at a point 5cm above the soil level using a Vernier caliper. The weekly increase in growth was graphically presented for each growth attribute.

By the end of 9th week after transplanting, the experiment was terminated. The mature maize plants were gently and carefully uprooted. The plant samples were sorted into roots, stems, leaves and fruits, washed with deionized distilled water and finally each sample was separately air dried. The soil samples were also

taken out from each of the pots; air dried and kept in sterile sampling bags by labeling each appropriately. The dried plant and soil samples were grinded separately and each of the ground samples was kept for metal contents analyses.

Digestion of soil and plant samples: The air dried soil samples from each of the mentioned treatment was crushed, ground and powdered with a mortar and pestle. Precisely, 0.2g of powdered soil was carefully weighed into a Teflon beaker and a mixture of 1ml nitric acid (HNO₃), 3ml perchloric acid (HClO₄) and 1ml hydrofluoric acid (HF) was added to the sample. The content was heated on a hot plate in a fume cupboard till colorless solution was formed. After cooling, the residue was transferred into 25ml volumetric flask and made volume up to the mark with deionized distilled water. Similarly, the plant samples i.e., root, stem, leaf and fruit of each treatment was crushed, ground and powdered separately with the help of a mortar and pestle. An amount of 0.2g powdered plant sample was carefully weighed into a Teflon beaker and a mixture of 1ml 70% perchloric acid (HClO₄), 5ml nitric acid (HNO₃) and 0.5ml sulphuric acid (H₂SO₄) was added to the sample. The content was heated on a hot plate in a fume cupboard till the appearance of a clear solution. It was then set aside to cool. The residue was transferred into 25ml volumetric flask and made the volume up to the mark with deionized distilled water. The digested samples were then analyzed for their various heavy metals (viz., Ni²⁺, Pb²⁺, Cu²⁺ and Cd²⁺) by using atomic absorption spectrophotometry (AAS). The absorbance for the determination of Ni²⁺,

Pb²⁺, Cu²⁺ and Cd²⁺ was recorded at wavelength of 232.0, 283.3, 324.8 and 228.8 nm, respectively. All plastic ware used during the experiments and for storage of reagents and standards were pre-cleaned with 20% HCl for 24 h, thoroughly rinsed with deionized water (18.2 M Ω cm⁻¹, Elgastat, Maxima, UK), stored in re-sealable plastic bags to prevent contamination and used as required. All reagents and standards were of analytical grade (Merck Dram Stad, Germany) unless otherwise stated. Standard stock solutions (100 mg L⁻¹) of Ni²⁺, Pb²⁺, Cu²⁺ and Cd²⁺ were prepared from atomic absorption standards (Spectrosol, BDH, UK) in 0.01M HCl, and various working standard solutions were prepared from these stock solutions by serial dilution with 0.01M HCl. Atomic absorption spectrometer (PYE Unicon SP-9) with single pin chart recorder (PM 8251, Phillips, Japan) was used for the determination of micronutrients. For atomization an air acetylene flame was used. The absorbance for the determination of each metal ion was recorded at different wavelengths. Similarly the digested samples of soil, root and shoot of potted maize were then separately analyzed for their aforementioned micronutrients.

Mycorrhizal fungal (*Glomus mosseae*) contribution to metal content of maize and underlying soil: Percentage arbuscular mycorrhizal fungal (i.e., *G. mosseae*) contribution to metal content of potted maize, and underlying soils in SS and DS pots containing crude-oil contaminated and uncontaminated (control) soils were calculated using the given formula:

For potted maize:

$$\% \text{ AM}_{\text{Contr.}} = \frac{\text{Nutrient concn., in inoculated maize} - \text{Nutrient concn., in uninoculated maize}}{\text{Nutrient concentration in uninoculated maize}} \times 100$$

For the underlying soil:

$$\% \text{ AM}_{\text{Contr.}} = \frac{\text{Nutrient concn., in inoculated soil} - \text{Nutrient concn., in uninoculated soil}}{\text{Nutrient concentration in uninoculated soil}} \times 100$$

Data analysis: Data obtained for various shoot growth attributes of potted maize plants subjected to different soil contamination and inoculation treatments were processed via Microsoft excel programme and the treatment means separated using standard error of means. The data collected for various heavy metal contents of the study were also statistically analyzed for working out their analysis of variance (ANOVA). The MSTAT-C computer software package, version 1.3 was used for the purpose mentioned (Steel & Torrie, 1980). The treatment means were separated by Duncan's Multiple Range Test @ p < 0.05.

Results

Growth attributes: Results exhibited that inoculation not only promoted the height of maize plants grown singly in normal soils (Fig. 1a), but also alleviated the inhibitory effect of crude oil contamination. By 7th week after planting (WAP), the growth performances of inoculated maize plants grown in oil contaminated soils (C⁺M⁺) have overtaken those of non-inoculated plants in control soils. Similar trends of increased plant height were also

observed in densely sown (DS) maize plants. However, the growth in height of C⁺M⁺ did not overtake the growth of plants in control soils (Fig. 1b). Results pertaining to weekly increases in stem girth exhibited the similar pattern as that of plant height (Fig. 2a & b).

Data obtained for leaf length showed that as the time progressed, the leaf length also increased up to 3 to 4 weeks after planting. Thereafter, a significant drastic reduction in all treatments both for SS and DS was recorded (Fig. 3a & b). A maximum leaf length was recorded for M⁺ plants in each case of experiment. It was also noted that DS plants comparatively produced longer leaf over SS grown in each treatment. Results also enumerated that as the time interval increases, leaf width (cm) also progressively increased up to 5 WAP. After that a drastic decline (up to 8 WAP) in all treatments (except control) were observed both in SS and DS potted plants (Fig. 4a & b). Data also showed that a maximum leaf width is noted for M⁺ and a minimum for control treatments in either set of experimental plants. It was further observed that DS plants produced greater leaf width over those of SS potted maize plants.

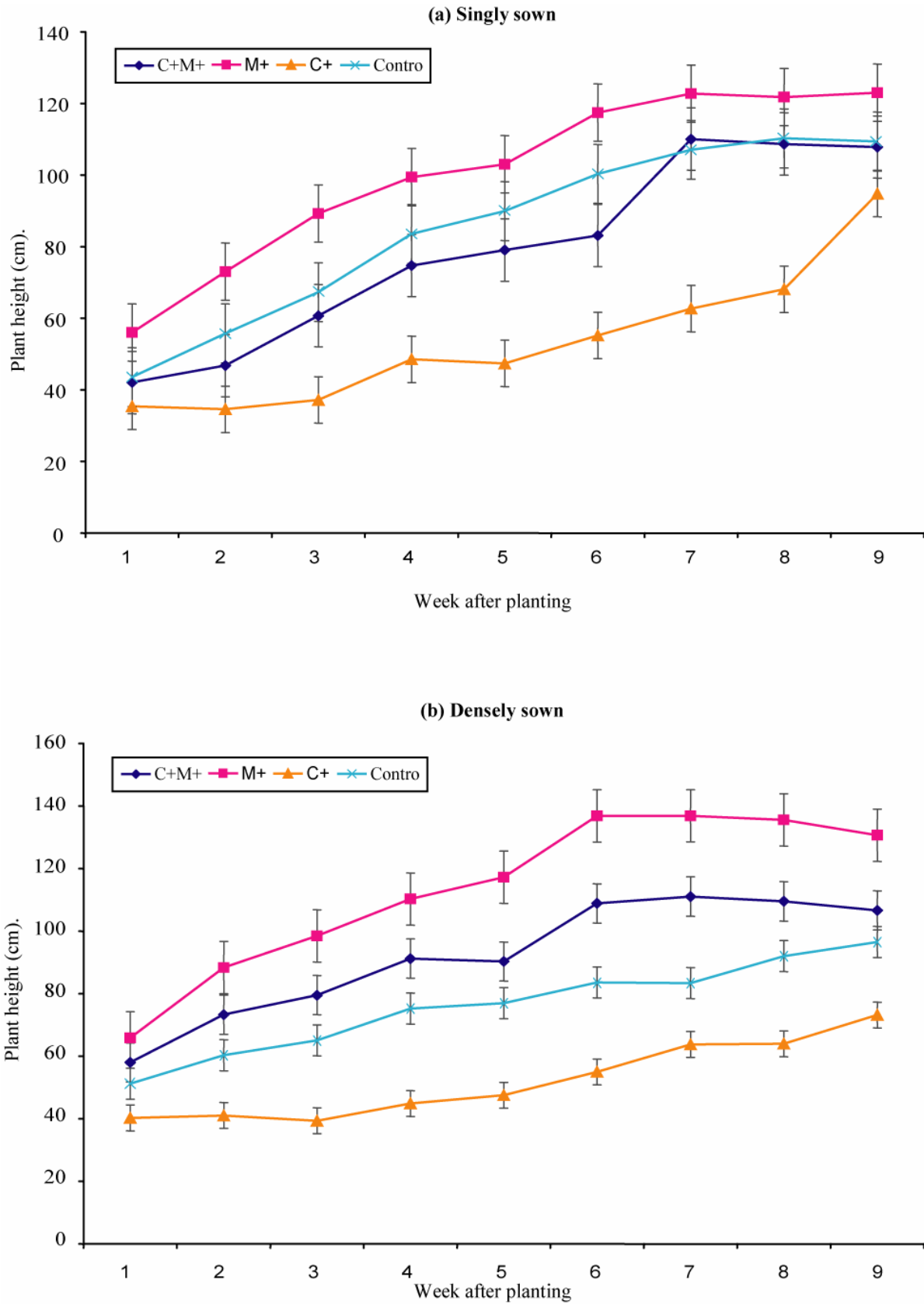


Fig. 1a & b. The effect of soil inoculation with *Glomus mosseae* and contamination with crude oil on weekly increase in plant height of singly and densely sown potted maize plants.

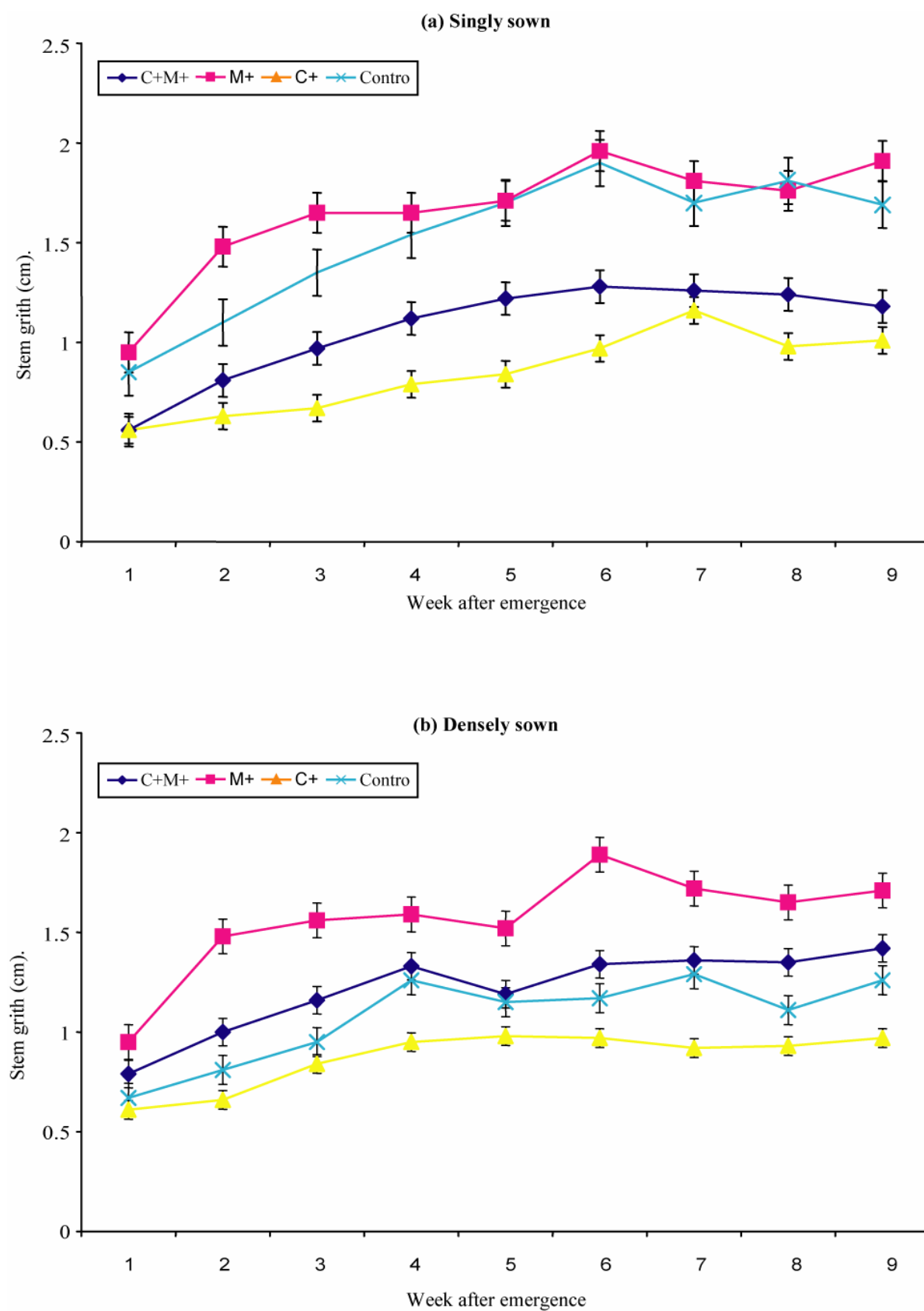


Fig. 2a & b. The effect of soil inoculation with *Glomus mosseae* and contamination with crude oil on weekly increase in stem girth of singly and densely sown potted maize plants.

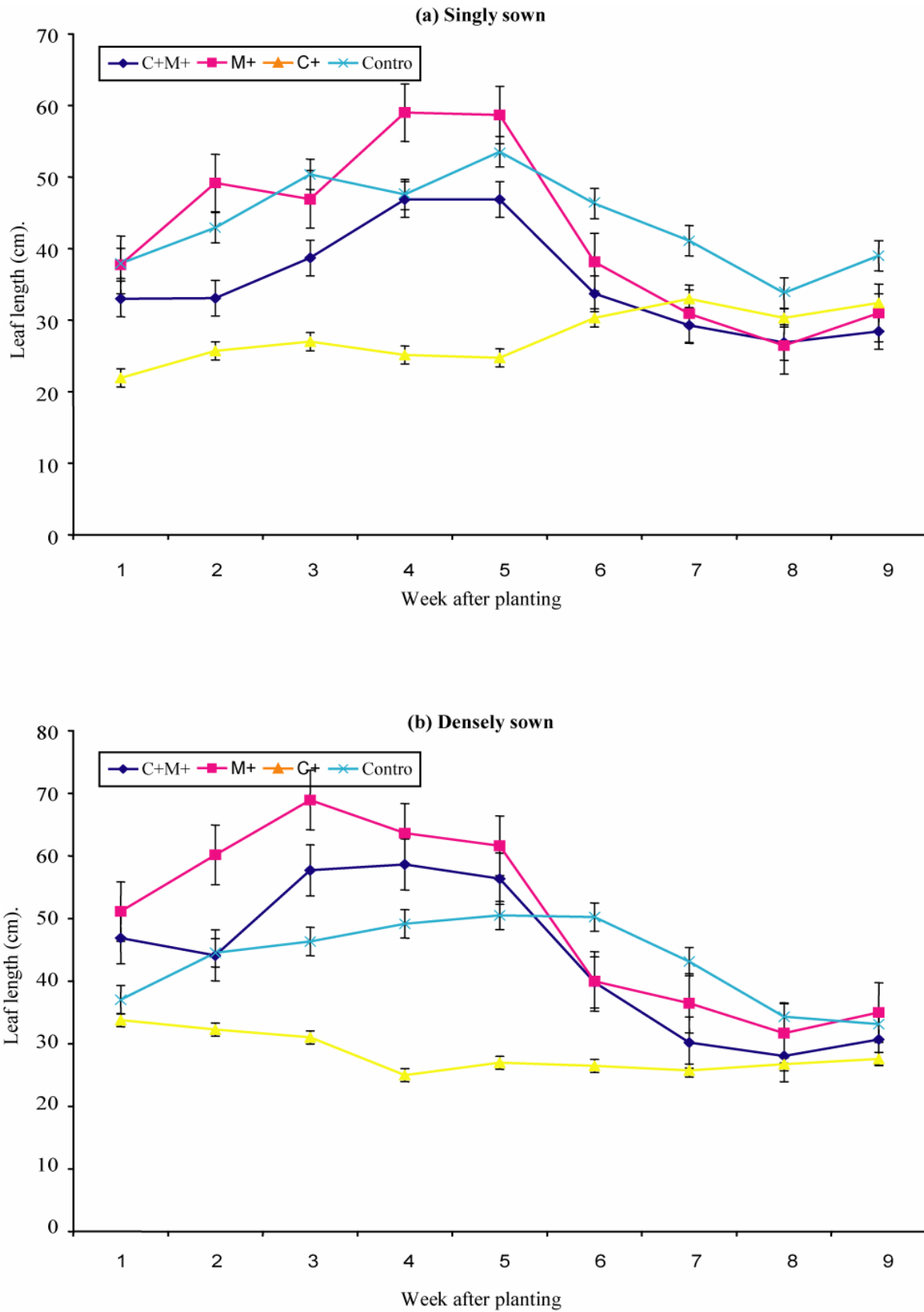


Fig. 3a & b. The effect of soil inoculation with *Glomus mosseae* and contamination with crude oil on weekly increase in leaf length of singly and densely sown potted maize plants.

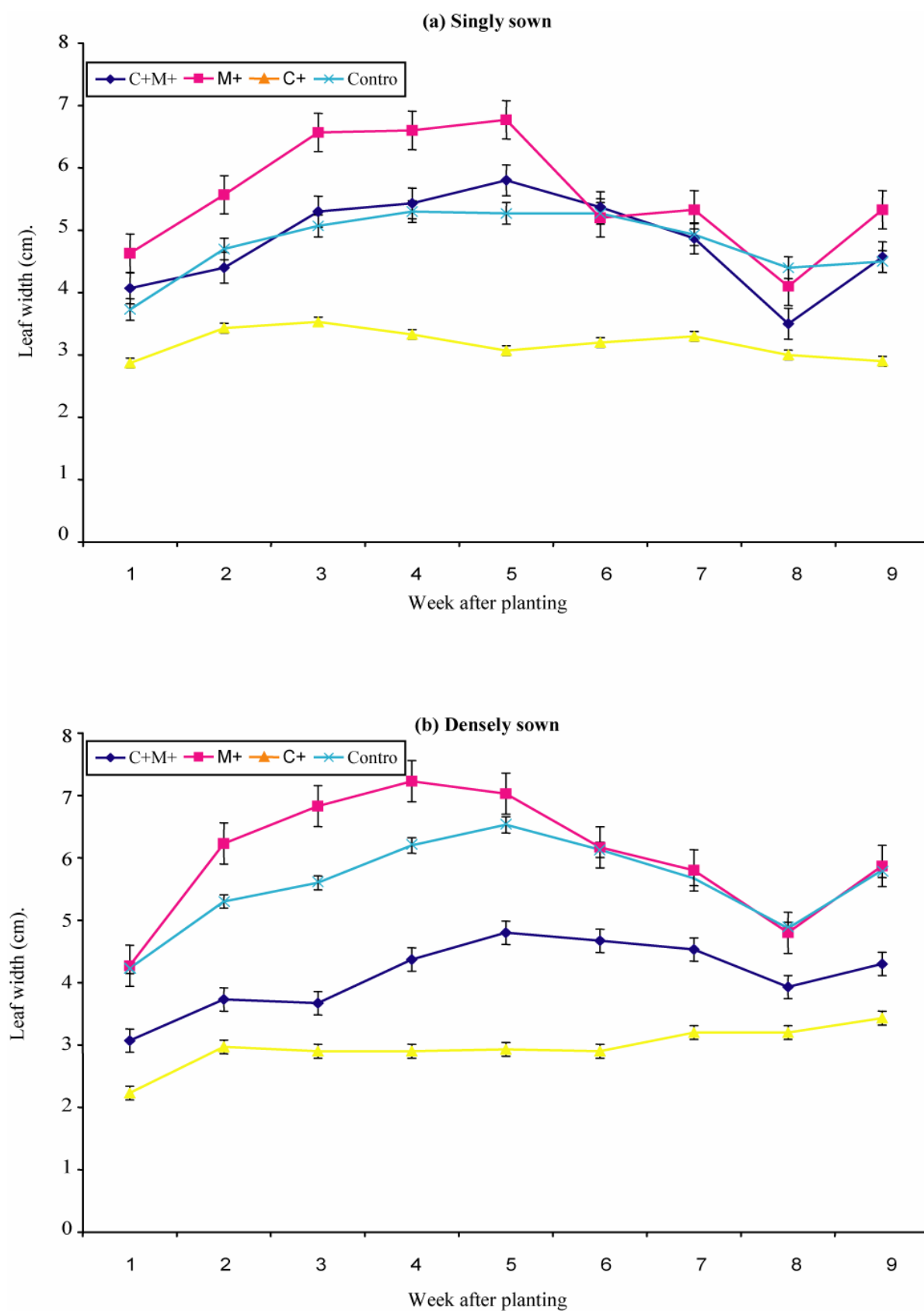


Fig. 4a & b. The effect of soil inoculation with *Glomus mosseae* and contamination with crude oil on weekly increase in leaf width of singly and densely sown potted maize plants.

Effect of *G. mosseae* on metal absorption and internal distribution in potted maize grown in contaminated soils: High planting density (DS) promoted extraction and storage of Ni²⁺ and Cu²⁺ in the roots of inoculated maize plants grown in C⁺M⁺, while those of Pb²⁺ and Cd²⁺ often remain unaffected by planting density (Table 1). The amount of metals in roots of inoculated maize plants was higher than non-inoculated, irrespective of crude-oil contamination or planting density. Maize plants grown in contaminated soils had higher root concentration of all metals than those grown in uncontaminated control soils. Inoculation with *G. mosseae* assisted phytoextraction of all

four metals from crude oil contaminated soils by maize plants irrespective of planting density. However, in the absence of mycorrhizal partnership, hyperextraction of metals from crude-oil contaminated soil was inhibited as most of the metals with exception of Ni²⁺ were found in the soil. The amounts of metals present in the soil/plant/above ground complex were generally low for inoculated/non-inoculated as well as SS/DS plants. In the absence of AM inoculation, planting density appears to reduce the level of metal ions in C⁺ soils and it actually promoted hyperextraction of Ni²⁺.

Table 1. The effect of crude oil contamination and AM inoculation on the concentration of selected heavy metals (mg kg⁻¹) in soil, root and shoot of singly sown (SS) and densely sown (DS) potted maize in a fixed volume of soil.

| Soil treatments | Sample source | Metal concentration (mg kg ⁻¹) in singly (SS) and densely planted (DS) pots. | | | | | | | |
|-------------------------------|---------------|--|--------|------------------|--------|------------------|--------|------------------|-------|
| | | Ni ²⁺ | | Pb ²⁺ | | Cu ²⁺ | | Cd ²⁺ | |
| | | SS | DS | SS | DS | SS | DS | SS | DS |
| C ⁺ M ⁺ | Soil | 123.1b | 34.2c | 54.5b | 99.3a | 151b | 100.4c | 6.3c | 5.5c |
| | Root | 119.3b | 198.3b | 18.6c | 19.1c | 109.8c | 137.9b | 21.9a | 19.5b |
| | Shoot | 443.7a | 367.5a | 128.6a | 68.9b | 393.1a | 411.2a | 17.9b | 21.2a |
| C ⁺ M ⁻ | Soil | 5.3a | 9.5a | 4.8a | 12.5a | 3.8a | 7.6a | 3.9a | 3.0a |
| | Root | 0.3c | 0.6c | 0.4c | 0.6c | 0.8c | 0.3c | 1.2b | 1.0b |
| | Shoot | 2.0b | 1.9b | 2.5b | 1.7b | 2.5b | 1.3b | 1.4b | 1.2b |
| C ⁻ M ⁺ | Soil | 99.4a | 58.6b | 161.7a | 127.2a | 133.6a | 87.4a | 15.8a | 17.9a |
| | Root | 6.8c | 8.4c | 5.4c | 5.8c | 2.4c | 3.4c | 0.4c | 0.5c |
| | Shoot | 14.7b | 87.7a | 10.9b | 20.0b | 20.2b | 29.9b | 2.1b | 12.6b |
| C ⁻ M ⁻ | Soil | 6.0a | 6.4a | 6.3a | 7.3a | 5.6a | 7.2a | 0.6a | 1.1a |
| | Root | 0.2c | 0.3c | 0.7c | 0.8b | 0.7b | 0.3c | 0.1b | 0.3c |
| | Shoot | 123.1b | 34.2c | 54.5b | 99.3a | 0.8b | 1.2b | 0.7a | 0.5b |

*C⁺M⁺ = Contaminated & inoculated, C⁺ = Contaminated & uninoculated, M⁺ = Uncontaminated & inoculated and Control = uncontaminated & uninoculated. ** Means within the same soil treatment followed by different letters are significantly different at p>0.05 according to Duncans Multiple Range Test (DMRT).

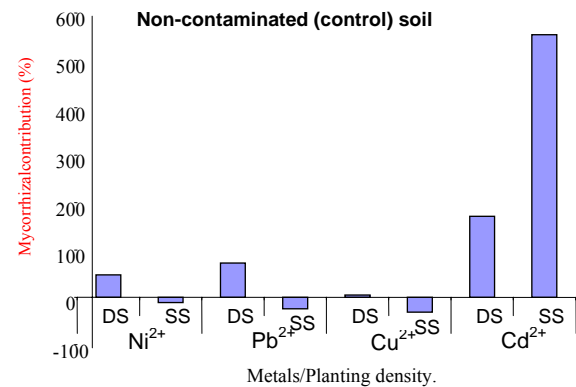
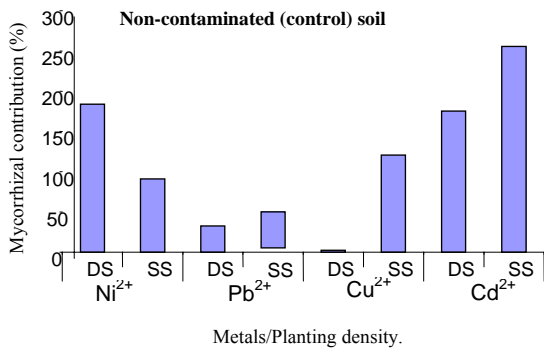


Fig. 5. Mycorrhizal contribution (%) to metal content of potted maize grown on control and crude oil contaminated soils.

Fig. 6. Mycorrhizal contribution (%) to metal content of control and crude oil contaminated soils under potted maize plants.

Mycorrhizal contribution to metal content of maize:

Inoculation with *G. mosseae* contributed positively to the concentration of the selected heavy metals in maize plants grown in C⁺M⁺ soils irrespective of planting density (Fig. 5). The highest contribution goes to Ni²⁺ content of SS maize plants, while the lowest were for Pb²⁺ & Cd²⁺ content of DS maize. However, for maize grown in control soils, the highest contribution of *G. mosseae* to metal content of maize was to the Cd²⁺ content of SS maize, while the lowest was in Cu²⁺ content of DS maize (Fig. 5).

Mycorrhizal contribution to metal content of soil:

Glomus mosseae contributed negatively to metal content of contaminated soil in all soil contamination and planting density treatments with the exception of Ni²⁺ of soil under SS maize and Cu²⁺ content of soil under DS & SS maize showed slightly positive contribution (Fig. 6). Meanwhile, in the control i.e., uninoculated soil, mycorrhizal contribution to soil under maize was low i.e., slightly negative or slightly positive as the case may be with the exception of AM contribution to Cd²⁺ content which is strongly positive particularly in soil under SS maize (Fig. 6).

Discussion

Results showed that the vegetative growth attributes of both DS and SS potted maize plants were more prolific in inoculated than uninoculated irrespective of soil contamination by crude oil. These observations are also in line with the results recorded by most of the other researchers in various crops inoculated with their respective inoculum e.g., maize (Liasu *et al.*, 2002; Ayotamuno & Kogbara, 2007; Ogbo *et al.*, 2010), tomato (Liasu, 2008) and amaranthus (Olusola & Anslem, 2010). Lasat (2002) also illustrated that increased capacity for phytoextraction as the amount of metals removed from the soil through phytoextraction by hyperaccumulating plants depend upon the above ground biomass production. Whereas, the least biomass production in the present study is recorded in C⁺. This clearly indicates that soil pollution by crude oil can impede the growth and metabolism of maize plants and the AM fungal inoculation could have encourage or speed up the biodegradation of various organic chemicals e.g., polycyclicaromatic hydrocarbons (PAHs) present in the crude oil thus releasing the useful mineral nutrients leading to a boost in the shoot growth and metabolism of the growing maize plants. Similar findings are also achieved by Liasu (2008) in tomato grown on soils amended with composted brewery waste.

The high Ni²⁺ concentration in above ground maize plant tissue followed by their roots in C⁺M⁺ could be attributed to the fact that Ni²⁺ in a growing medium i.e., soil is capable of displacing most nutrients in the plant tissue. Da-Silva *et al.*, (2006) also stated that AM fungi increased the capacity of plants to extract contaminant from soil. Similarly, Shevyakova *et al.*, (2011) reported that exogenous application of putrescine increases Ni²⁺ accumulation in rape shoots, improving potential for phytoremediation of contaminated soil.

The low Pb²⁺ content in maize had been attributed to low availability of Pb²⁺ in soils. That the Pb²⁺ exclusion by AM inoculation reported by Liasu *et al.*, (2006) did not occur in singly planted maize grown in oil contaminated soils suggests that Pb²⁺ absorption with the exception of passive transport may be governed by the same

mechanism as cadmium's. Generally, hyperextraction of the selected metals from the soil is more favored by planting density in uncontaminated soils, while AM inoculation tends to exclude heavy metals from potted plants. However, in case of contaminated soils, AM inoculation promotes hyperextraction of metals more than planting density while the combination of the two phenomena act synergistically to promote metal hyperextraction from crude oil contaminated soils as well as uncontaminated soils. These findings are also in line with those of by Mathur *et al.*, (2007).

The Cu²⁺ content in the tissue of inoculated maize grown in oil contaminated soil (C⁺M⁺) accumulated the highest concentration in the shoot followed by root and soil when compared with remaining treatments, respectively. However, AM inoculation alone did not significantly improve the phytoaccumulation of Cu²⁺. Nwaichi *et al.*, (2010) reported that *Vigna subterranean* extracted up to 88.88 mg kg⁻¹ into its shoot and root respectively at 10% contaminant dose while achieving 63.17% Cu²⁺ removal unamended. Mathur *et al.*, (2007) also postulated that mycorrhiza enhances the uptake of Cu²⁺. On the contrary, Galli *et al.*, (1995) reported that no differences in Cu²⁺ uptake were detected between mycorrhizal and non-mycorrhizal plants. Therefore, present studies results do support the idea that AM fungi protects maize from Cu-toxicity.

The pattern of Cd²⁺ distribution appears to favor the assumption of earlier reporters (Mathur *et al.*, 2007) that its uptake process is a passive one driven by concentration gradient irrespective of inoculation and planting density. However, Cd²⁺ exclusion particularly in the uncontaminated control soil is probably mediated by AM inoculation through the agency of fungal hyphae which adsorb the metal and keep it sequestered on active sites of the hyphal wall (Lasat, 2002). On the contrary, the observed promotion of Cd²⁺ extraction from oil contaminated soils by inoculated maize could be as a result of the saturation of the active sequestration sites thus subjecting the absorption process to simple laws of diffusion (Lasat, 2002). The concentration gradient is also maintained through chelation-induced Cd²⁺ release as a result of increased root exudates production under AM symbiosis (Ebbs *et al.*, 1997). Whereas, Gianinazzi *et al.*, (2002) narrated that *Glomus* sp., increased Cd²⁺ concentrations in clover roots, but not in shoots and also did not affect the plant growth.

Conclusion

Inoculation of potted maize plants with arbuscular mycorrhizal fungus (*Glomus mosseae*) promoted the vegetative growth both in crude oil contaminated and uncontaminated (control) soils. AM inoculation promoted the hyperextraction of heavy metals from oil contaminated soils, but inhibited their phytoextraction from uncontaminated soils. High planting density promoted the phytoextraction of heavy metals from uncontaminated soils but had minimal effect on phytoextraction from oil contaminated soils. Thus planting density complemented the promotory effect of AM inoculation on phytoextraction of heavy metals from crude oil contaminated soils.

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