Phytoremediation of Cadmium Contaminated Soil by Indian Mustard and Spinach as Influenced by Phosphate Fertilizer and Mycorrhizae

THESIS

Submitted to the

Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior

In partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE
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By

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CERTIFICATE – I

This is to certify that the thesis entitled **‘Phytoremediation of cadmium contaminated soil by Indian mustard and spinach as influenced by phosphate fertilizer and mycorrhizae’** submitted in partial fulfilment of the requirement for the **DEGREE OF MASTER OF SCIENCE (Soil Science and Agricultural Chemistry)** of the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior is a record of the bonafied research work carried out by **Mr. Sandeep Karode ID.No.RA/IN/846/2009** under my guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee and the Director of Instruction.

No part of the thesis has been submitted for any degree or diploma (Certificate awarded etc.) or has been published. All the assistance and help received during the course of the investigation has been acknowledged by the scholar.

Place: Sehore　　　　　　　　　　Signature

Date:　　　　　　　　　　　　　**(Dr. R.C. Jain)**

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Member (Dr. S.C. Gupta) ………………………….

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CERTIFICATE –II

This is to certify the thesis **“Phytoremediation of cadmium contaminated soil by Indian mustard and spinach as influenced by phosphate fertilizer and mycorrhizae”** submitted by **Mr. Sandeep Karode ID.No.RA/IN/846/2009** to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfillment of the requirements for the degree of Master of Science in **AGRICULTURE (Soil Science & Agricultural Chemistry)** has been accepted after evaluation by the External Examiner and approved by the Student’s Advisory Committee after an oral examination on the same.

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Date: Chairman of the Advisory Committee

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Legend
&	And
@	At the rate of
^{o}C	Degree Celsius
%	Percentage
C.D.	Critical Difference
Cd	Cadmium
cm	Centimeter
C.V.	Coefficient of Variation
cv	Cultivar
DAS	Days after sowing
d.f.	Degree of Freedom
DHA	Dehydrogenase activity
et al.	And others
etc	and the rest
FDA	Fluorescein di-acetate
fig.	Figure (s)
g	Gram
ha	Hectare
i.e.	That is
K	Potassium
kg	Kilogram (s)
kg/ha	Kilogram per hectare
l	Litre
MSS	Mean sum of square
mg	Miligram
m	Meter (s)

N	Nitrogen
no	Number (s)
NS	Non significant
P	Phosphorus
S	Sulphur
SMBC	Soil microbial biomass carbon
SR	Soil respiration
R.V.S.K.V.V.	Rajmata Vijaya Raje Scindia Krishi Vishwa Vidyalaya
R.A.K.	Rafi Ahmed Kidwai
RH	Relative humidity
S.Em.±	Standard error of mean
S.S.	Sum of Square
Viz.	Namely
√	Square root
%	Percent
±	Plus or Minus

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CHAPTER I
INTRODUCTION

Pollution of the natural environment by heavy metals is a growing concern because these metals are indestructible and most of them have toxic effects on living organisms present in the soil ecosystem. Heavy metals frequently reported in literature with regards to potential hazards and occurrences in contaminated soils are cadmium (Cd), chromium (Cr), lead (Pb), zinc (Zn), iron (Fe) and copper Cu (Alloway, 1995; Akoto *et al.,* 2008). Increasing levels of heavy metals contamination caused by anthropogenic activity mainly with vehicle exhausts as well as several industrial activities (Ghrefat and Yusuf, 2006). The accumulation and transfer of heavy metals in the soil-plant-animal system is cause food chain contamination (Wright and Welbourn, 2002; Landis and Yu, 2003: Bradl, 2005;). Besides industrial activities, the excessive fertilizer use in agriculture also has great contribution in heavy metals contamination. The content of heavy metals in fertilizers is generally as follows: phosphatic fertilizer> compound fertilizer> potash fertilizer> nitrogen fertilizer (Boyd, 2010). Nevertheless, the long-term excessive application of agricultural inputs mainly phosphatic fertilizers and pesticides resulted in the heavy metal contamination of soils. Among different heavy metals, Cd is one of the most hazardous heavy metal and show deleterious effects on agricultural ecosystem, environment and human health. The presence of Cd in soil is not only harmful for the living communities (soil organisms, plants and animals), it also affects the biogeochemical cycle which are necessary for maintenance of life. Its presence has been detected in various water bodies, groundwater, agricultural soil and various foods consumed by humans. Increased concentrations of Cd in agricultural soils are known to come from human activities (Taylor, 1997), such as the application of phosphate fertilizer, sewage sludge, wastewater, and pesticides (Chen *et al.,* 1997; Qadir *et al.,* 2000; de Meeus *et al.,* 2002; Kara *et al.*, 2004), mining and smelting of metalliferous ores with high Cd content (Tembo *et al.,* 2006; Kovacs *et al.*, 2006), and traffic (Nabulo *et al.,* 2006).

Cadmium concentrations above the threshold limit values (give value) have been found to be carcinogenic, mutagenic and teratogenic for a large number of animal species (Degraeve, 1981). The most severe form of chronic Cd poisoning is known as *Itai-itai* disease (Ishihara *et al.*, 2001; Inaba *et al.*, 2005). Cadmium is water soluble and can be transferred efficiently from soil to plants, which may affect human health if there is excessive intake from a contaminated food source (Satarug *et al.*, 2003).

Bioremediation technique has been used for the remediation of soils polluted with heavy metals. Song *et al.* (1996) reported that the accumulation of heavy metals in the edible parts of spinach (*Spinacia oleracea* L.) depended on the doses, status of elements in the soil and interaction with an accumulation rate order: Cd>Zn>Pb. The roots of Indian mustard are found to be effective in the removal of Cd, Cr, Cu, Ni and Pb (Lone *et al.*, 2008). Indian mustard (*B. juncea*) is a high biomass, rapidly growing plant that has an ability to accumulate Cd in its shoots. In addition, mycorrhizal colonization reduced Cd accumulation in pea shoots (*Pisum sativum* L.) when no Cd was added to the soil, but increased and decreased Cd accumulation in shoots and pea pods, respectively, when 100 mg Cd kg-1 was added to the soil as $CdCl_2$ (Rivera Becerril *et al.*, 2002). In some cases mycorrhizal plants can show enhanced heavy metals uptake and root-to-shoot transport (phytoextraction) while in other cases mycorrhizal fungi contribute to heavy metals immobilization within the soil (phytostabilization) (Gaur and Adholeya 2004; Khan, 2005). The various metal tolerant mycorrhizal fungi have which are found to be evolved as a trace metal-tolerance and thus they may play important role in the phytoremediation of the site (Liao *et al.,* 2003; Gohre and Paszkowski, 2006; Owska *et al.,* 2011; Zarei *et al.*, 2010). Concern regarding this latter route (agricultural crops) led to research on the possible consequences of applying sewage sludge (Cd-rich biosolids) to soils used for crops meant for human consumption, or of using Cd enriched phosphate fertilizer (Campbell, 2006). Abul Kashem and Kawai (2007) have reported that 10 mM Mg alleviated the 0.25 μM Cd toxicity in Japanese mustard and spinach plants and exhibited two-fold higher shoot growth with decreased shoot Cd concentration of 40%.

In the first and third crops of spinach, cadmium uptake increased with increasing cadmium concentration in soil. However, the dry matter was not significantly affected, except during the third crop at 50 μg g^{-1} cadmium level (Darshana Salaskar *et al*., 2011).

Bioremediation is the process of utilizing living organisms to reduce or eliminate the hazardous chemicals accumulated in the soil. The predominant organisms used are bacteria, fungi, algae, plankton, protozoa, and plants. Naturally occurring organisms, as well as genetically modified ones, can potentially be used. Organisms can destroy organic chemicals but they can also either remove or convert metals to a stable form. The basic principles behind bioremediation are bioaccumulation, biosorption, and biocrystalisation. Bioremediation using plants is known as phytoremediation (Evangelou, 1998).

The phytoremediation of Cd with spinach and mustard has been reported elsewhere but none of the studies revealed the story with how phosphate fertilization along with mycorrhizal application improved this techniques.

Keeping alone aims in view, the present study entitled ‘Phytoremediation of cadmium contaminated soil by Indian mustard and spinach as influenced by phosphate fertilizer and mycorrhizae’ was carried out with the following objectives-

1. To know the phytoremediation potential by the Indian mustard on cadmium contaminated soil and cadmium accumulation in spinach.
2. To know the effect of single super phosphate and arbuscular mycorrhizae on Cd phytoremediation.
3. To study about the effect of phytoremediation on soil microbiological activities and nutrient availability.

CHAPTER II

REVIEW OF LITERATURE

1. History of phytoremediation

The basic idea that plants can be used for environmental remediation is quite old. The knowledge that aquatic or semi-aquatic vascular plants, water hyacinth (*Eichhornia crassipes*), pennyworth (*Hydrocotyle umbellata*), duckweed (*Lemna minor*), and water velvet (*Azolla pinnata*), can take up Pb, Cu, Cd, Fe and Hg from contaminated solutions existed for a long time. Some Italian researchers first reported nickel (Ni) hyperaccumulation in the Italian serpentine plant *Alyssum bertolonii* in 1948. This finding was all but forgotten until 1977, when researcher Robert Brooks, of Massey University in New Zealand, made similar observations. Later, on the morning of April 29, 1986, a nuclear explosion occurred in a small town in the former Sovier Union. It caused severe radioactive contamination in area up to 100 km from site and it literally shook the earth. Three years after the explosion (1989), the Soviet government asked the International Atomic Energy Agency (IAEA) to assess the radiological and health situation in the area surrounding the power plant. The IAEA had surveyed the area and found radioactive emission and toxic metals-including iodine, cesium-137, strontium, and plutonium concentrated in the soil, plants and animals. These toxic substances entered the food chain via grazers, such as cows and other livestock that fed on plants grown in contaminated soils. To prevent further spread of these toxins, it was determined that livestock should be allowed to feed only on uncontaminated plants and on plants not tending to accumulate toxic metals within their tissues. The scientists were hopeful that plants may play a key role in cleaning up some of the contamination. Then a soil cleanup method was employed using green plants to remove toxins from the soil. This technique is phytoremediation, a term coined by Dr. Ilya Raskin of Rutgers University’s Biotechnology Center for Agriculture and the Environment, who was a member of the original task force sent by the IAEA to examine food safety at the Chernobyl site. The word phytoremediation come from the Greek word phyto, meaning ‘plant’ and the Latin word remedare, meaning ‘to remedy’. It has been defined as the use of green plants and their associated micro-organisms, soil amendments and agronomic techniques to remove. Contain or render harmless environmental contaminants. It also called as “Green remediation” and “Botanical bioremediation”. It represents a typical in-situ biological treatment, whose main advantage is that it allows the soil to be treated without being excavated and transported, resulting in potentially significant cost savings. Today, many researchers, institutes, and companies are funding scientific efforts to test different plants effectiveness at removing a wide range of contaminants.

Sustainable approaches to land remediation, which include the use of plants and microbes to transform or uptake toxic substances, have been proposed. These techniques have not been widely applied due to time restraints (Cost and risk), the identification of species that are appropriate for remediation and the cost in the case of use of microbes (Chen *et al.*, 2004). As much as these remediation technologies (both sustainable and unsustainable) have shown promise for individual pollutants (Gao and Zhu, 2004), it has not been the case for sites that are contaminated by more than one single pollutant. This has been the challenge for soil remediation as many contaminated sites do not contain one single pollutant but instead a number of different substances (Lin *et al.*, 2008; Zhang *et al.*, 2011). Therefore combinations of traditional techniques are used to remediate these soils, which of late have included methods that use microbes. This combination of techniques uses energy in many cases and with the emphasis on sustainability, low energy and environmentally friendly technologies are required and phytoremediation could solve this problem.

Phytoremediation is the use of green plants and their associated microbes to remove environmental pollutants or to render them harmless (Kambhampati and Vu, 2013). It is a plant-based technology that enhances environmental clean-up (Pilon-Smiths, 2005; Cook and Hesterberg, 2013). It has the advantage of being less expensive than most traditional methods (the fact that it is carried out in-situ), environmentally sustainable since solar energy drives the process and aesthetically pleasing (Singer *et al.*, 2007). However phytoremediation has limitations: for example, it is limited to sites where contamination is low and confined to rooting depth and the remediation process could affect the food chain when chemicals are degraded or taken up by plants (Pilon-Smiths, 2005). Phytoremediation makes use of natural processes where the plants in combination with their microbial rhizosphere degrade and take up pollutants (organic and inorganic). It has shown to be effective in clean up of both organic and inorganic pollutants (Pilon-Smiths, 2005). Most organic pollutants that are released into the environment are anthropogenic and are xenobiotic to organisms showing evidence of toxicity and carcinogenicity (Meagher, 2000). The uptake or degradation of organics by plants is dependent on the pollutant properties such as their recalcitrant nature and solubility in water (Smith *et al.*, 2006). Some organics such as Trichloroethylene (TCE) (Newman *et al.*, 1997, Lewis *et al.*, 2013), the herbicides atrazine (Burken and Schnoor, 1997; Murphy and Coats, 2011), petroleum hydrocarbons (Schnoor *et al.*, 1995; Cook and Hesterberg, 2013), polychlorinated biphenyls (Harms *et al.*, 2003; Li *et al.*, 2013) have been successfully removed using plants.

1.1. Definition of Phytoremidiation :-

The generic term ‘phytoremediation’ consists of the Greek prefix phyto (plant), attached to the Latin root remedium (to correct or remove an evil) (Cunningham *et al.*, 1996). Phytoremediation is an alternative or complimentary technology that can be used along with or, in some cases in place of mechanical conventional clean-up technologies that often require high capital inputs and are labour and energy intensive. Phytoremediation is an *in-situ* remediation technology that utilizes the inherent abilities of living plants. It is also an ecologically friendly, solar-energy driven clean-up technology, based on the concept of using nature to cleanse nature (UNEP, Undated). Contamination of soil by oil spills is a wide spread environmental problem that often requires cleaning up of the contaminated sites (Bundy *et al.*, 2002).

Phytoremediation is a broad term that has been used since 1991 to describe the use of plants to reduce the volume, mobility, or toxicity of contaminants in soil, groundwater, or other contaminated media (USEPA, 2000). Plants can help clean up many kinds of pollution including metals, pesticides, explosives, and oil. The plants also help prevent wind, rain, and groundwater from carrying pollutants away from sites to other areas. Phytoremediation is a non-destructive and cost effective in-situ technology that can be used for the cleanup of contaminated soils. The potential for this technology in the tropics is high due to the prevailing climatic conditions which favours plant growth and stimulates microbial activity (Zhang *et al.*, 2010).

For at least 300 years, the ability of plants to remove contaminants from the environment has been recognized and taken advantage of applications such as land farming of waste. Over time, the use of plants has evolved to the construction of treatment wetlands or even the planting of trees to counteract air pollution. In more recent years, as recognition grew of the damage resulting around the world from decades of an industrial economy and extensive use of chemicals, so did interest in finding technologies that could address the residual contamination, among them phytoremediation (USEPA, 2000).

1.2. Mechanisms of Phytoremediation

The mechanisms and efficiency of phytoremediation depend on the type of contaminant, bioavailability and soil properties (Cunningham and Ow, 1996). There are several ways by which plants clean up or remediate contaminated sites. The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing toxicity are found. The root system provides an enormous surface area that absorbs and accumulates water and nutrients essential for growth along with other non-essential contaminants (Raskin and Ensley, 2000).

1.2.1. Phytoextraction

To remove contamination from the soil, this approach uses plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the above ground biomass (shoots, leaves, etc.). Discovery of metal hyperaccumulator species demonstrates that plants have the potential to remove metals from contaminated soils (Raskin and Ensley, 2000). A hyperaccumulator is a plant species capable of accumulating 100 times more metal than a common non-accumulating plant (UNEP, Undated). Metals such as nickel, zinc and copper are the best candidates for removal by phytoextraction because it has been shown that they are preferred by a majority of plants (approximately 400) that absorb unusually large amounts of metals.

The use of hyperaccumulator species is limited by slow growth, shallow root system, and small biomass production. In addition, the plant biomass must also be harvested and disposed of properly, complying with standards (Raskin and Ensley, 2000). There are several factors limiting the extent of metal phytoextraction including:

- Metal bioavailability within the rhizosphere
- Rate of metal uptake by roots
- Proportion of metal “fixed” within the roots
- Rate of xylem loading/translocation to shoots
- Cellular tolerance to toxic metals

The method is also usually limited to metals and other inorganic compounds in soil or sediment (EPA, 2000).

1.2.2. Rhizofiltration

This is primarily used to remediate extracted groundwater, surface water, and wastewater with low contaminant concentrations. It is the adsorption or precipitation onto plant roots or absorption of contaminants in the solution surrounding the root zone. Rhizofiltration is typically exploited in groundwater, surface water, or wastewater for removal of metals or other inorganic compounds (EPA, 2000). The advantages associated with rhizofiltration are the ability to use both terrestrial and aquatic plants for either in-situ or ex-situ applications. Another advantage is that contaminants do not have to be translocated to the shoots. Thus, species other than hyper accumulators may be used. Terrestrial plants are preferred because they have a fibrous and much longer root system, increasing the amount of root area (Raskin and Ensley, 2000).

1.2.3. Phytovolatilization

This involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere (USEPA, 2000). Phytovolatilization also involves contaminants being taken up into the body of the plant, but then the contaminant, a volatile form thereof, or a volatile degradation product is transpired with water vapor from leaves (EPA, 2000).

Phytovolatilization may also entail the diffusion of contaminants from the stems or other plant parts that the contaminant travels through before reaching the leaves (Raskin and Ensley 2000). Phytovolatilization can occur with contaminants present in soil, sediment, or water. Mercury is the primary metal contaminant that this process has been used for. It has also been found to occur with volatile organic compounds, including tri-chloroethene, as well as inorganic chemicals that have volatile forms, such as selenium, and arsenic (EPA, 2000).

1.2.4. Phytostabilization

It is the use of certain plant species to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (rhizosphere). This process reduces the mobility of the contaminant and prevents migration to the ground water and it reduces bio-availability of metal into the food chain. This technique can also be used to re-establish vegetation cover at sites where natural vegetation fails to survive due to high metals concentrations in surface soils or physical disturbances to surface materials. (EPA, 2000).

1.2.5. Phytodegradation

This is also referred to as phytotransformation. It involves the degradation of complex organic molecules to simple molecules or the incorporation of these molecules into plant tissues (Trap *et al.*, 2005). When the phytodegradation mechanism is at work, contaminants are broken down after they have been taken up by the plant. As with phytoextraction and phytovolatilization, plant uptake generally occurs only when the contaminants' solubility and hydrophobicity fall into a certain acceptable range. Phytodegradation has been observed to remediate some organic contaminants, such as chlorinated solvents, herbicides, and munitions, and it can address contaminants in soil, sediment, or groundwater (EPA, 2000).

1.2.6. Rhizodegradation

This is also referred to as phytostimulation. Rhizodegradation refers to the breakdown of contaminants within the plant root zone, or rhizosphere. It is believed to be carried out by bacteria or other microorganisms whose numbers typically flourish in the rhizosphere. Studies have documented up to 100 times as many microorganisms in rhizosphere soil as in soil outside the rhizosphere (USEPA, 2000). It can also be seen as plant-assisted bioremediation, the stimulation of microbial and fungal degradation by release of exudates/enzymes into the root zone (rhizosphere) (Zhuang *et al.*, 2005).

2. Importance of phytoremediation

Garbisu (2002), defined phytoremediation as an emerging cost effective, non-intrusive, aesthetically pleasing, and low cost technology using the remarkable ability of plants to metabolize various elements and compounds from the environment in their tissues. Phytoremediation technology is applicable to a broad range of contaminants, including metals and radionuclides, as well as organic compounds like chlorinated solvents, polychloribiphenyls, polycyclic aromatic hydrocarbons, pesticides, insecticides, explosives and surfactants.

According to Macek (2004), phytoremediation is the direct use of green plants to degrade, contain, or render harmless various environmental contaminants, including recalcitrant organic compounds or heavy metals. Plants are especially useful in the process of bioremediation because they prevent erosion and leaching that can spread the toxic substances to surrounding areas.

According to Moffat (1995), research has been slow and tedious due to scientists' incomplete understanding of the generalized cellular mechanisms of plants. However, the advent of new genetic technology has allowed scientists to determine the genetic basis for high rates of accumulation of toxic substances in plants.

According to Huang and Cunningham (1996), using genetic engineering, scientists may soon be able to exploit plants' characteristics that can provide faster and more efficient means of removing contaminants from the soil. Genetic engineering will also be crucial for the creation of transgenic plants that will be able to combine the natural agronomic benefits associated with plants (ease of harvest and rapid, expansive growth) with the remediation capabilities of bacteria-a traditional organism used in bioremediation.

This process is extremely expensive and, therefore not entirely appealing despite recent discoveries regarding phytoremediation (Lasat, 2000). Analysts have estimated the cost of cleaning one hectare of highly contaminated land at a depth of one meter. The cost of phytoremediation could be as much as 20 times less expensive, making this practice far less prohibitive than conventional methods (Lasat, 2000).

Table 1: General advantages and disadvantages of Phytoremediation (Raskin and Ensley, 2000)

Advantages	Disadvantages
Cost	**Time**
Low capital and operating cost	Slower compared to other techniques and seasonally dependent
Metal recycling provides further economic advantages	Most of the hyperaccumulators are slow growers
Performance	**Performance**
1) Permanent treatment solution.	1) Not capable of 100% reduction.
2) *In-situ* application avoids excavation.	2) May not be functional for all mixed wastes.
3) Capable of remediating bio available fraction of contaminants.	3) Soil phytoremediation is applicable only to surface soils.
Other	**Other**
1) Public acceptance due to aesthetic reasons.	1) Regulators are unfamiliar with this new technology.
2) Compatible with risk-based remediation. Can be used for site investigation or after closure.	2) Lack of recognized economic performance data.

Initially, waste disposal into the environment was based on the assumption that pollutants will be absorbed. However, emphasis on the need for sustainable and efficient treatment technologies has resulted from the accumulation of toxic pollutants and deterioration of global environmental health, loss of biodiversity and water resources, ecosystem imbalance and impairment, poor vegetation development or complete habitat destruction and adverse health effects with increase in morbidity and mortality (Bamforth and Singleton, 2005; Conte *et al.*, 2005; Escalante-Espinosa *et al.*, 2005; Scullion, 2006; Paria, 2008; Glick, 2010).

On the whole, phytoremediation offers the advantage of a cost-effective approach, solardriven technology with minimal energy requirements, minimal environmental disruption, preservation of biological activity in soils, improvement of soil microbial diversity, long term applicability, application to a range of contaminants and public acceptance compared to the traditional methods (Morikawa and Erkin, 2003; Dheri, *et al.*, 2007; Zhuang *et al.*, 2007; Vangronsveld *et al.*, 2009; Wenzel, 2009).

The physical, chemical and thermal processes are the common techniques that have been involved in the cleaning up of oil contaminated sites (Frick *et al.*, 1999). These techniques however have some adverse effects on the environment and are also expensive (Frick *et al.*, 1999; Lundstedt, 2003). Recently, biological techniques like phytoremediation are being evaluated for the remediation of sites contaminated with petroleum. Phytoremediation is the use of plants and associated microorganisms to remove contain or render harmful material harmless (Cunningham *et al.*, 1996; Schwab and Banks, 1999; Merkl, 2005). It has been shown to be effective for different kinds of pollutants like heavy metals, radionuclides and broad range of organic pollutants (Schroder *et al.*, 2002; Schnoor, 2002) According to Pivetz (2001), plants for phytoremediation should be appropriate for the climatic and soil conditions of the contaminated sites. Such plants should also have the ability to tolerate conditions of stress (Siciliano and Germida, 1998a).

3. Cadmium contamination in the soil water systems

3.1. Chemistry of cadmium and its use

In its elemental form, cadmium is a silver-white coloured, lustrous metal that can be easily cut by a knife at room temperature (MERCK, 2006; ATSDR, 2008). Cadmium metal will slowly oxidise in moist air to form cadmium oxide, which is a greenish-yellow, brown through red to black crystalline solid or powder (MERCK, 2006; ECB, 2007).

Cadmium has a similar chemistry to zinc with a strong affinity for sulphur (Alloway, 1995; Greenwood and Earnshaw, 1997; Kabata-Pendias and Mukherjee, 2007). Its compounds almost exclusively involve the 2^+ oxidation state and may be highly coloured (Greenwood and Earnshaw, 1997). Cadmium forms simple salts with oxygen, sulphur and many common anions including chloride, nitrate and carbonate (Greenwood and Earnshaw, 1997). In aqueous solution, cadmium often forms simple hydrated hxdroxyl ions such as $[Cd(OH)(H2O)x]^+$ it also has an appreciable coordination chemistry with legends' including halides, hydroxides, cyanides and nitrate, although the range is much less extensive than for other transition metals (Alloway, 1995; Greenwood and Earnshaw, 1997)

Cadmium has also been used in pigments for plastics, glass and ceramics, as a fungicide, in stabilisers for plastics including PVC, and in corrosion-resistant coatings on steel and other non-ferrous metals; it has other minor applications for photography, photocopying, dyeing, calico printing, vacuum tube manufacture, galvanoplasty, lubricants, ice-nucleation agents, and in the manufacture of special mirrors (Alloway, 1995; ECB, 2007; Kabata-Pendias and Mukherjee, 2007; ATSDR, 2008).

3.2. Potential harm to human health

The principles behind the selection of Health Criteria Values (HCVs), and the definition of concepts and terms used, are outlined in Human health toxicological assessment of contaminants in soil (Environment Agency, 2009c). Specific information on the toxicity of cadmium and its compounds is reviewed in Contaminants in soil: updated collation of toxicological data and intake values for humans. Cadmium (Environment Agency, 2009d) and only a brief summary are presented here.

Following long-term exposure to cadmium, the main health concerns are its toxicity to the kidney and bones, arising via ingestion and inhalation, and its lung carcinogenicity seen in exposed workers following inhalation (Environment Agency, 2009d).

3.3. Exposure assessment

3.3.1 Occurrence in soil

Cadmium occurs naturally in soils as a result of the weathering of the parent rock (Alloway, 1995). Sedimentary rocks have the greatest range of cadmium concentrations with the highest values found in sedimentary phosphate deposits and black shales (Alloway, 1995). Although most natural soils contain less than 1 mg kg^{-1} cadmium from the weathering of parent materials, those developed on black shales and those associated with mineralised deposits can have much higher levels (Alloway, 1995).

Anthropogenic sources of cadmium are much more significant than natural emissions and account for its ubiquitous presence in soil (Alloway, 1995; ECB, 2007; ATSDR, 2008). Cadmium is a trace element in phosphatic fertilizers, which have been applied extensively to arable and pasture land in the UK for decades (Alloway, 1995). ECB (2007) reported that current fertilizers contain around 79 mg of cadmium per kilogram of phosphorus. Based on the use of fertilizers in the 1980s and early 1990s, Alloway (1995) estimated that around 4.3 g of cadmium per hectare per year has been added to agricultural soils in the UK.

Atmospheric deposition is also an important source of cadmium pollution (Alloway, 1995; ECB, 2007; ATSDR, 2008). The major sources of atmospheric emissions are non-ferrous metal production, fossil fuel combustion, waste incineration, and iron and steel production (Alloway, 1995; ATSDR, 2008). A representative deposition rate to agricultural land across the European Union has been estimated to be 3 g of cadmium per hectare per year (Alloway, 1995; Kabata-Pendias and Mukherjee, 2007). Other sources of cadmium contamination include the application of sewage sludge, and metalliferous mining and smelting of zinc and sulphide ores (Alloway, 1995; ECB, 2007; ATSDR, 2008).

3.3.2 Behaviour in the soil

Several studies have observed that cadmium concentrates in the surface horizon, most likely due to the combination of the sources of pollution (primarily fertilizers application and atmospheric deposition) and the higher near-surface levels of organic matter to which it readily binds (Alloway, 1995; ATSDR, 2008).

Surface adsorption processes rather than precipitation appear to control the distribution of cadmium between soil solution and soil-bound forms at the concentrations relevant to most polluted soils (Alloway, 1995). At extremely high cadmium concentrations, however, the precipitation of phosphates and carbonates could be expected (Alloway, 1995). Under anaerobic conditions, cadmium in soil solution may be controlled by the formation of insoluble sulphides (Kabata-Pendias and Mukherjee, 2007).

Adsorption of cadmium by soil depends strongly on pH, with its mobility decreasing with increasing alkalinity (Anderson and Christensen, 1988; Alloway, 1995; Holm *et al.*, 2003). Soil organic matter (SOM) is also an important factor (Holm *et al.*, 2003; Kabata-Pendias and Mukherjee, 2007). Sauve *et al.* (2000) found that the variability in soil–water partition coefficients for cadmium collected from over 70 different studies was largely explained by differences in soil pH, soil organic matter content and total cadmium concentration. Strobel *et al.*, (2005) observed that anthropogenic cadmium may be more readily released from soil than pedogenic cadmium associated with soil minerals such as apatite.

Organic matter may be better at keeping cadmium unavailable than specific and non-specific adsorption to inorganic mineral surfaces (Holm *et al.*, 2003; ATSDR, 2008). Strobel *et al.*, (2005) noted that the addition of dissolved organic carbon did not directly enhance the release of cadmium from a Danish agricultural soil but assisted in decreasing the soil pH, which resulted in more rapid cadmium leaching. A secondary factor controlling the mobility of cadmium is the presence of iron and manganese hydrous oxides, and organic matter–iron complexes (Alloway, 1995; Holm *et al.*, 2003; Kabata-Pendias and Mukherjee, 2007).

Plant species and cultivars differ widely in their ability to uptake, accumulate and tolerate heavy metals including cadmium (Alloway, 1995; Environment Agency, 2009e). Lettuce, spinach, celery and cabbage are reported to accumulate cadmium while potatoes, french beans and peas take up only small amounts (Alloway, 1995). Several studies have concluded that soil pH is the most important factor controlling plant uptake and that this is linked to the concentration of cadmium in soil solution (Kabata-Pendias and Pendias, 2001; ATSDR, 2008; Environment Agency, 2009e).

4. Role of phosphorus for cadmium dynamics in soil plants system

Phosphorus (P) is an essential nutrient for crop production and growth. For soils deficient in P, application of organic or inorganic fertilizers is needed to achieve optimum crop yields. Although P fertilizers represent the major anthropogenic input of P to agricultural soils, both inorganic P fertilizers and organic P sources, such as sewage sludge and manure, may contain Cd (Sheppard *et al.*, 2009). In addition to direct inputs of Cd, phosphate fertilization can indirectly affect Cd accumulation in crops through its effects on soil chemistry, crop growth, and microbial interactions. Therefore, the management of P fertilizer application, both in the short- and long-term, can influence the potential accumulation of Cd in foods.

The Cd present in the phosphate rock source remains throughout the fertilizer production process, leading to a large range of Cd concentrations in P fertilizers from different sources. As the amount of Cd added to soils from P fertilizer application is a function of the rate and frequency of application, and the concentration of Cd in the fertilizer material, the total input, and the net Cd balance will vary widely depending on the fertilizer source and the crops grown. However, both the input and output of Cd tend to be low relative to the total amount of Cd present in the soil (Christensen and Tjell, 1991; Christensen and Huang, 1999; Sheppard *et al.*, 2009).

In greenhouse studies, Cd concentrations in carrot (*Daucus carota,* L.), lettuce (*Latuca sativa,* L.), oat (*Avena sativa,* L.), and ryegrass (*Lolium multiflorum,* L.) were increased by application of NPK fertilizer containing 417 mg Cd kg^{-1} P, but not by addition of low-solubility rock phosphate containing a similar Cd concentration (He and Singh, 1995).

The long-term effects of P fertilizer application are also of concern because the Cd added into agricultural systems in P fertilizers accumulates over time if application rates are in excess of Cd removal. Long-term trials in Sweden (Andersson, 1977), Denmark (Dam Kofoed and Sondergard-Klausen, 1983; Christensen and Tjell, 1991), Norway (Baerug and Singh, 1990), Britain (Jones *et al.*, 1987; Nicholson and Jones, 1994) Finland (Makela-Kurtto *et al.*, 1991), and the United States (Mulla *et al.*, 1980) have clearly demonstrated that a cumulative surplus Cd balance leads to gradual increases in soil Cd concentrations over time. The use of P fertilizers with low Cd concentrations has been examined in several long-term field trials. For example, in nine long-term soil fertility experiments in the United States, the application of recommended rates of P fertilizers containing an estimated 5 mg Cd kg^{-1} for more than 50 years increased soil Cd concentrations by between 0% and 0.5% per year (Mortvedt, 1987).

As the Cd concentration of crops is strongly affected by soil Cd concentration (Adams *et al.*, 2004), long-term increases in Cd in the soil resulting from P application can lead to higher crop Cd accumulation (Williams and David, 1973; Williams and David, 1976; Mulla *et al.*, 1980; Jones *et al.*, 1987; Jones and Johnston, 1989; Hamon *et al.*, 1998; Kashem and Singh, 2002; Brennan and Bolland, 2004).

In addition to direct inputs of Cd, phosphorus fertilizer can indirectly affect Cd accumulation in crops through its effects on soil chemistry, crop growth, and microbial interactions (Grant, 2011). When we apply phosphorus fertilizer to our cropland, we are also feeding trace element (mainly Cd) which after certain time lead to its accumulation in our cropland soil (Grant and Sheppard, 2008; Jiao *et al.*, 2012). Plants take up Cd with water and 2 nutrients when grown in Cd contaminated soil (Akhter, 2012).

Loganathan *et al.*, (1995) showed a clear relationship between the amount of phosphorus (P) fertilizer use and Cd accumulation in plants. Cadmium can be present in phosphate fertilizer at concentrations ranging from 0 to 300 mg kg^{-1}, depending on the provenance of the phosphate rock (Mortvedt *et al.*, 1981). (Andersson and Siman, 1991) and (Grant and Bailey, 1997), the management of P fertilizer application, both in the short term and long term, can influence the potential accumulation of Cd in foods. Hence it is important to minimize Cd accumulation in agricultural soils.

4.1 Phosphorus Fertilizer

Nziguheba and Smolders, (2008), was reported that the commercial fertilizer had played a major role to ful fill increasing demand of world food production. Because of the more extended use of fertilizer and their great importance for crop production, more studies and attention also had been paid to understand and quantify the potentially toxic risk of the trace elements contained in them. These elements can get into the soil-plant system and enter into the foodstuffs. Concentrations of those potentially toxic hazardous elements are largely dependent on the raw materials used to produce them. In phosphate fertilizer the raw material used is known as phosphate rock.

Dylevskaia, (2002), was reported that the types of raw material used for phosphate fertilizer is divided in two types, (i) the phosphate sedimentary rocks such as shales and pelitic types, accounts for 88% of total phosphate rock production, and is mainly found in North Africa, especially in countries such as Morocco and 5 Tunisia, and (ii) the igneous rocks like basalts, are mainly produced in Russia and South Africa.

4.2 Soil Cadmium

Cadmium was discovered in 1817 by Strohmeyer of Germany, who isolated it from calamine (zinc carbonate). The name cadmium is derived from cadmia, the ancient Greek name for calamine (Nriagu, 1980). Cadmium is a non-essential, potentially toxic element for both plants and animals. It is highly mobile and bioavailable in the environment (McLaughlin and Singh, 1999).

4.3 Cadmium in Soils and Plants

The major sources of Cd in soils are atmospheric emissions from mining and direct application of phosphate fertilizers, sewage, sludge, manure and composted municipal solid waste on agricultural soils, and accidental contamination from industrially contaminated land and mine waste dumps (Diskshith and Diwan, 2003). But among all sources, Cd containing phosphate fertilizers are a major source of anthropogenic Cd in agricultural systems (Sheppard *et al.*, 2007; Grant and Sheppard, 2008; Grant, 2011). These fertilizers may contain Cd as a contaminant at levels ranging from 0 mg kg^{-1} to as high as 340 mg kg^{-1} on a total dry weight basis (Alloway and Steinnes, 1999). Therefore, along with total Cd, the bioavailable fraction of Cd in the soil is also important in determining Cd toxicity to plants.

Bioavailable forms of Cd in soil include free Cd^{2+}, Cd^{2+} organic ligands (Cd^{2+}organic acids, Cd^{2+}humate), Cd^{2+} inorganic ligands ($CdCl^{+}$,$CdOH^{+}$). The ability to release Cd^{2+} from these complexes in the soil system depends on a number of factors including soil pH (Peijnenburg *et al.*, 2000), organic matter (Murray *et al.*, 2011), cation exchange capacity (Bolan *et al.*, 2003a, 2003b), presence of competing or complexing ions (Gao *et al.*, 2011), and crop management practices (Gao *et al.*, 2010).

4.4 Plant Uptake

Increasing soil cadmium concentration will commonly be associated with their increased accumulation in crops (Adams *et al.*, 2004). Huang *et al.*, (2004), Jones *et al.*, (2002) found fertilized soils had significantly lower levels of available and total metals than those of non-fertilized soils. He and Singh (1994) compared the Cd uptake by oats, ryegrass, carrot and spinach grown on sandy and loamy soils that were amended with Cd salt, low Cd NPK fertilizer, high Cd NPK fertilizer, or rock phosphate at comparable amounts of Cd input. The plants grown with the high Cd NPK fertilizer accumulated the most Cd. In the sandy soil, the Cd uptake by oats and ryegrass fertilized by the high Cd NPK fertilizer was two times more than those grown in control soil. For the phosphate rock treatment, the Cd contents in the plant tissue were not significantly different from those in plants grown on the control soil.

In a long-term field investigation, Hamon *et al.*, (1998) showed that the Cd contents of field grown wheat increased as corresponding amounts of superphosphate applied increased. Huang *et al.*, (2004) reported that less than 1% of the Cd added through fertilizer application accumulated in the lettuce biomass.

6.5 Indirect Effects of P Fertilizers on Cd Availability in Soils

In contaminated soils, the application of high rates of phosphate may attenuate the phytoavailability of Cd in soils by P-induced Cd^{2+} adsorption or formation of less soluble metal-phosphate minerals or precipitates. The increased adsorption of Cd^{2+} could be a function of surface complex formation on P compounds, increased surface charge, or co-adsorption of P and Cd as an ion pair (McGowen *et al.*, 2001; Bolan and Duraisamy, 2003; Carrillo-Gonzalez *et al.*, 2006).

Carrillo-Gonzales *et al.*, (2006) suggested that since the free activities of Cd^{2+} in soil solutions are far below the potential chemical equilibrium, the solubility and mobility of Cd in soils is likely controlled by sorption or coprecipitation mechanisms. The effectiveness of phosphate addition in reducing Cd bioavailability tends to be greater with more soluble sources (Basta and McGowen, 2004). In pot studies, high applications of P as finely ground rock phosphate or KH_2PO_4 to an industrially contaminated soil reduced Cd concentrations in sorghum-sudan grass (*Sorghum bicolor,* L. Moench) (Zwonitzer *et al.*, 2003). However, in other studies, application of fused and superphosphate (FSP) at rates of up to 400 mg P kg^{-1} increased Cd availability of radish (*Raphanus sativus,* L.), while further increasing the application rate of FSP to over 800 mg P kg^{-1} resulted in decreased availability (Hong *et al.*, 2008).

Phosphorus fertilizers may indirectly influence Cd bioavailability by reducing the pH of the soil solution. Soil pH is a major factor influencing Cd solubility and mobility in soils (Carrillo-Gonzalez *et al.*, 2006; Rieuwerts *et al.*, 2006), with solubility and ion activity decreasing with increasing pH, and Cd sorption increasing (Naidu *et al.*, 1994; Carrillo-Gonzalez *et al.*, 2006).

The release of ammonium from mono-ammonium phosphate and di-ammonium phosphate may also lead to displacement of Cd from adsorption sites, thereby increasing bioavailability (Lorenz *et al.*, 1994; Mitchell *et al.*, 2000; Lambert *et al.*, 2007).

5. Use of mycorrhizae in phytoremediation

In addition, mycorrhizal colonization reduced Cd accumulation in pea shoots (*Pisum sativum,* L.) when no Cd was added to the soil, but increased and decreased Cd accumulation in shoots and pea pods, respectively, when 100 mg Cd kg^{-1} was added to the soil as $CdCl_2$ (Rivera Becerril *et al.*, 2002). In flax (*Linumu sitatissimum,* L.), application of P fertilizer or planting the crop after cultivating a non-mycorrhizal canola (*Brassica napus,* L.) crop rather than after spring wheat (*Triticam. aestivum,* L.) was associated with increased Cd concentrations, a finding that is possibly related to reduced mycorrhizal colonization (Grant *et al.*, 2010). However, another study found no relationship between mycorrhizal colonization and Cd accumulation in durum wheat. Therefore, while the application of P fertilizers may decrease mycorrhizal colonization, which possibly affects Cd accumulation, further research is required to more clearly understand these interactions.

5.1 Mycorrhizal Associations

Mycorrhizae are the symbiotic relationship between a soil-borne fungus and the roots of a plant. Mycorrhizal fungi depend on host plants for carbon and in return they enable host plants to be more efficient in acquiring nutrients and water from the soil (Entry *et al.*, 1999). There are two types of mycorrhizal fungi: ectomycorrhizae and endomycorrhizae. Ectomycorrhizae form sheaths around plant roots (Rost *et al.*, 1998). Endomycorrhizae enter cortex cells in the plant roots (Rost *et al.*, 1998).

Inoculating potted plants in the nursery, such as tree seedlings, with specific mycorrhizae may also be a good idea if a specific type of mycorrhizal fungi is desired since some types have been shown to work better than others (Entry 2 *et al.*, 1994). Inhibition of mycorrhizal growth can occur due to high phosphate levels in the soil (Killham, 1995).

5.2 Heavy metal-tolerant AM fungi

Heavy metals have been reported to reduce or eliminate AM infection at high concentrations of heavy metals in the soil (Koomen *et al.*, 1990), thus interfering with possible beneficial effects of the mycorrhizal association.

The metal tolerant AM isolates decrease the metal absorption capacity and filter the intake of metal ions in plants (Martina and Vosatka, 2005). The various metal tolerant mycorrhizal fungi have which are found to be evolved as a trace metal-tolerance and thus that they may play important role in the phytoremediation of the site (Liao *et al.*, 2003; Gohre and Paszkowski, 2006; Orowska *et al.*, 2011; Zarei *et al.*, 2010).

In recent years several studies have shown the harmful effects of metals on microbial diversity and activity in soil (Citterio *et al.*, 2005 and Glassman and Casper, 2012).High concentrations of trace metals in soil have an adverse effect on microorganisms. The accumulation of metals in soils at high concentrations can be due to anthropogenic activities such as application of sewage sludge. Mycorrhizal fungi that provide a direct link between soil and roots may be of great importance to plants growing in soils contaminated with trace metals (Leyval *et al.*, 1997).

AM spore population decreased with increased amount of trace metals in the soil (Val *et al.*, 1999; Hayes *et al.*, 2003). The mycorrhizal root colonization of AMF fungi were found decreased by the higher levels of trace metals in the soil. Our results also supports the findings of (Shah *et al.*, (2010); Biro *et al.*, (2005); Mathur *et al.*, (2007).

A comparative study of response of a plant in soil inoculated with arbuscular mycorrhizal fungi (AMF) and non-AMF was adopted to investigate the sequence of some physiological and biochemical changes and factors that may interfere with tolerance mechanism of plants during uptake of heavy metals (Harrier and Watson 2003).

5.3 Role of mycorrhizae in phytoremediation

Mycorrhizal fungi, which form a symbiotic relationship with the roots of approximately 95% of vascular plant species, provide a direct physiological connection between the soil and plant roots (Smith and Read, 1997). Specifically, these fungi enhance the ability of plants to acquire nutrients from the soil (Barea and Jeffries, 1995; Smith and Read, 1997) and improve the resistance of plants to biotic and abiotic stresses (Azcon-Aguilar and Barea, 1996; Auge, 2001; Borowicz, 2001; Graham, 2001). Thus, plant roots infected with mycorrhizal fungi benefit from increased growth and fitness (Koide *et al.*, 1988; Stanley *et al.*, 1993; Poulton *et al.*, 2001; Lekberg and Koide, 2005 and references within). In addition to transferring nutrients from the soil to plants, mycorrhizal fungi may also transfer (or prevent the transfer of) cadmium from the soil to the plant (Gaur and Adholeya, 2004), thereby enhancing (or mitigating) plant responses to soil cadmium.

The extramatrical fungal hyphae can extend several cm into the soil and uptake large amounts of nutrients, including heavy metals, to the host root. The effectiveness of AM root colonization in terms of nutrient acquisitondi€ers markedly between AM fungi and host plant genotype (Ahiabor and Hirata, 1995; Marschner, 1995).

Mycorrhizae have also been reported in plants growing on heavy metal contaminated sites (Shetty *et al.*, 1995; Weissenhorn and Leyval, 1995; Pawlowska *et al.*, 1996; Chaudhry *et al.*, 1998; Chaudhry *et al.*, 1999) indicating that these fungi have evolved a HM-tolerance and that they may play a role in the phytoremediation of the site. Noyd *et al.* (1996) reported that AM fungal infectivity of native prairie grasses increased over three seasons on a coarse taconite iron ore tailing plots which helped to establish a sustainable native grass community that will meet reclamation goals.

Various authors have reported isolating spores of arbuscular mycorrhizal fungal texa such as *Glomus* and *Gigaspora* associated with most of the plants growing in heavy metal polluted habitats (Raman *et al.*, 1993; Raman and Sambandan, 1998; Chaudhry *et al.*, 1999). Pawlowska *et al.* (1996) surveyed a calamine spoil mound rich in Cd, Pb and Zn in Poland and recovered spores of *Glomus aggregatum, G. fasciculatum* and *Entrophospora spp.* from the mycorrhizospheres of the plants growing on spoil. Galli *et al.*, (1994) suggested that mycorrhizae can play a crucial role in protecting plant roots from heavy metals. Joner and Leyval (1997), reported that extra-radical hyphae of AM fungus *G. mosseae* can transport Cd from soil to subterranean clover plants growing in compartmented pots, but that transfer from fungus to plant is restricted due to fungal immobilization. The authors also reported no restriction of fungal hyphal growth into soil with high extractable Cd levels. Turnau (1998) studied the localization of heavy metals within the fungal mycelium and mycorrhizal roots of *Euphorbia cyparissias* from Zn contaminated wastes and found higher concentrations of Zn as crystaloids deposited within the fungal mycelium and cortical cells of mycorrhizal roots. Studies by various researchers (Galli *et al.*, 1994; Hetrick *et al.*, 1994; Leyval *et al.*, 1995) have shown that mycorrhizal fungal ecotypes from heavy metal contaminated sites seem to be more tolerant to heavy metals (and have developed resistance) than reference strains from uncontaminated soils.

5.4 Mycorrhiza-assisted remediation (MAR)

MAR is an aspect of bioremediation that uses mycorrhiza for the treatment of polluted soils. Mycorrhiza is the symbiotic association between fungi and the roots of vascular plants. The plant supplies the fungi with carbohydrate, while the fungi - known as mycorrhizal fungi - extends the surface area of the plant’s roots and thus increases their ability to absorb more nutrients (especially phosphorus) and water from the soil. Mycorrhiza increases the plant’s ability to resist diseases (Harrier and Watson, 2004). It also provides a stable soil for plant growth via production of glomalin - a substance that binds soil aggregates (Wright *et al.*, 2007).

Mycorrhiza cannot exist without a plant; therefore MAR can be described as a modified form of phytoremediation that exploits the benefits derived from mycorrhizal fungi. It uses some of the techniques of phytoremediation such as phytoextraction and phytostabilization. However, it is different from phytoremediation because remediation can be achieved at a faster rate since the area covered by plant roots - through the fungi hyphae - in MAR is larger than the area covered in phytoremediation (Gao *et al.*, 2010)

Rufyikiri *et al.*, (2004) observed that MAR reduced the translocation of pollutants from the roots to the shoots of plants. Thus, MAR increases the secondary value of plants used for phytoremediation (especially phytoextraction) because the plants that would normally be harvested and incinerated could be used to check erosion on the remediated soil. Furthermore, as the fungi spores remain in the soil for up to six years (Nguyen *et al.*, 2012), they easily colonize and support the growth of any crop planted on the soil after remediation. Thus, MAR ensures the rapid vegetation of remediated soils.

6. Phytoremediation of cadmium by using different crops

The obtained results allowed the selection of some species particularly prone to extract heavy metals from soil, i.e. sunflower, maize, mustard, barley, and pumpkin. In combination with soil amendment some agronomic crops might be used for the clean-up of contaminated soil. Cadmium is a mobile element, easily absorbed by roots and transported to shoots. It is uniformly distributed in plant organs while in the case of the poorly mobile elements (i. e. lead) their level decreases in the following order: roots > shoots > leaves > fruits > seeds. This distribution is due to the mobilization of the protective mechanisms of plants, which inhibits the transport to further tissues and organs. (Sekara A. *et al.* 2004)

Initially, the scope of phytoremediation was limited, principally because of the low bioavailability of heavy metals and the low biomasses of plants. Moreover, the management of the plant matter obtained after phytoremediation was troublesome (Gruca*et al.*, 2006; Nouairi *et al.*,2006; Zhao and McGrath, 2009). The success of phytoremediation depends mainly on the choice of plant, which must obviously possess the ability to accumulate large amounts of heavy metals (hyperaccumulation). These plants also have to satisfy other criteria:

1. The concentration of heavy metals in the shoots should be 50–100 times greater than in 'normal' plants (Jabeen *et al.*, 2009)
2. The bioaccumulation coefficient must have a value greater than 1 (McGrath, and Zhao., 2003)
3. Metal concentrations in the shoots should be higher than in the roots (Jabeen *et al.*, 2009)
4. Fast growth and high accumulating biomass (Marchiol *et al.*, 2004)
5. Easily grown as an agricultural crop and fully harvestable (Marchiol *et al.*, 2004)

Over 400 plant species have been identified as natural metal hyperaccumulators representing about 0.2% of all angiosperms. Unfortunately, most of these plants are characterized by slow growth and limited biomass production. It can be stated that the most frequently cited species in phytoremediation studies was *Brassica juncea* (L.), Czern (148 citations), followed by *Helianthus annuus* L. (57), *Brassica napus* L. and *Zea mays* L. (both 39 citations). The greater interest in *Brassicaceae* derives from the fact that research on these species started earlier, together with the interesting concentrations they provide, especially for *Brassica juncea* (L.) Czern (Vamerali,*et al.*, 2010).

It has been found that *Brassica juncea* exhibits a high capacity to accumulate Cd mainly in the shoots, where Cd level was recorded at level of 1450 µg Cd g^{-1} dry wt. This is three times more than reported in *Brassica napus* (555 µg g dry wt^{-1}) (Nouairi,*et al.*, 2006).

During testing capacity of phyoextraction of Zn, Cu and Pb for three *Brassica* crop species: *B. oleracea* L., *B. carinata* A. Br. and *B. juneca* (L.) Czern, the highest concentration of Zn (381 mg kg^{-1} dry wt.) and Cu (8.34 mg kg^{-1} dry wt.) were recorded in the shoots of *B. oleracea* L. The Pb concentrations of all *Brassica species* were more or less constant over the tested range of soil Pb concentrations, with lower values than the other metals. The low bioaccumulation of lead is due to its extreme insolubility and not generally being available for plant uptake in the normal range of soil pH (Gruca*et al.*, 2006; Gisbert *et al.*, 2006).

Among the Cd hyperaccumulators, *S. nigrum* L., *Populus spp,* Salix'calodendron' and Arabispaniculata (Wei *et al.*, 2005; French *et al.*, 2006; Maxted *et al.*, 2007), may be good candidates for field conditions due to their potentially higher biomass. Furthermore, their extraction capacity may be enhanced if suitable strategies of maximizing phytoremediation are adopted, based on knowledge of good agronomic practices and management (Keller *et al.*, 2003; McGrath *et al.*, 2006).

The idea of using plants to extract metals from contaminated soil was reintroduced and developed by (Utsunamyia, 1980) and (Chaney, 1983) and the first field trial on Zn and Cd phytoextraction was conducted in (Baker *et al.*, 1991).

Vegetable crop plants have high ability to accumulate metals from the environment, which may pose risks to human health when they are grown on or near contaminates lands and consumed. Metal accumulation in plant depends on plant species, growth stages, types of soil and metals, soil conditions, weather and environment (Asami, 1981; Chang *et al.*, 1984; Khairiah *et al.*, 2004).

Cadmium was found to be accumulated more in the roots of potato and onion (44 and 35 μg g^{-1} dry weight) followed by stem and leaves of potato, spinach roots and leaves, amaranthus leaves, fenugreek roots, and mustard leaves, while cluster bean, peas, carrot and soybean crops registered its lower accumulation. On an average Cd content was found highest in roots, followed by leaves, stem and fruits of vegetable crops (Singh *et al.*, 2012).

7. Indian mustard and spinach for cadmium uptake

Availability of K protects mustard plants from Cd toxicity by reducing its availability thereby depressing H_2O_2 content and lipid peroxidation, and increasing the activity of antioxidative enzymes (Umar *et al.*, 2008).

The up-regulation of S-assimilation pathway alleviates Cd toxicity and improves capacity of plants to survive in a Cd polluted environment (Wangeline *et al.*, 2004). Studies have indicated that both ATP-sulfurylase (ATPS) and serine acetyl transferase (SAT) played important roles in limiting Cd accumulation and enhancing Cd tolerance in Triticumaestivum (Khan *et al.*, 2007), *B. juncea* (Umar *et al.*, 2008), Arabidopsis (Howarth *et al.*, 2003).

Anjum *et al.*, (2008) have reported that S applied at 40 $mg{\cdot}S{\cdot}Kg^{-1}$ soil to mustard resulted in reduced Cd toxicity by increasing leaf AsA and GSH content.

In contrast, AbulKashem and Kawai (2007), have reported that 10 mM Mg alleviated the 0.25 μM Cd toxicity in Japanese mustard spinach plants and exhibited two-fold higher shoot growth with decreased shoot Cd concentration of 40%.

Qureshi *et al.*, (2010) observed that the supplementation of Indian mustard with 40 μM Fe suppressed oxidative stress and helped in retention of chloroplasts and chlorophylls and stabilizing thylakoid complex under Cd stress.

MD. AbulKashema and Shigenao Kawai, (2007) observed that To the best of our knowledge, information on Cd–Mg interactions in food crops is lacking. Japanese mustard spinach (*Brassica rapa* L. var. pervirdis) is one of the most common leafy green vegetables in most countries of the world. It is a fast grower and it is easy to detect toxic effects of surplus amounts of metals in the growth medium. Therefore, the objective of the present study was to investigate the effect of Mg on Cd toxicity with respect to growth and mineral nutrient status in Japanese mustard spinach grown in a nutrient solution.

The present study was conducted to determine the uptake and accumulation of cadmium in vegetable crops, spinach (*Spinacia oleracea* L.) and fenugreek (*Trigonella foenum* L.). Spinach plants did not show toxicity effects like chlorosis and necrosis due to various doses of cadmium at any stage of growth in both the first and third spinach crops in the sequence. In the first and third crops of spinach, cadmium uptake increased with increasing cadmium concentration in soil. However, the dry matter was not significantly affected, except during the third crop at 50 $\mu g\ g^{-1}$ cadmium level (Darshana Salaskar et al, 2011).

Spinach is an important vegetable crop consumed worldwide. A plant concentration of more than 100 μg g^{-1} cadmium may be regarded as exceptional; even on a cadmium-contaminated site. (Reeves and Baker, 2000)

Seeds of two cultivars of baby leaf spinach, Racoon and Donkey (RijkZwaan Australia), were sown into separate pots. Baby leaf spinach was used as the plant model as it is a known Cd accumulator (Liang *et al.*, 2013), is a small plant with rapid vegetative growth and the leaves and petioles are eaten in salads (Rogers *et al.*, 2008).

CHAPTER III

MATERIALS AND METHODS

The materials and methods related to the study on ‘Phytoremediation of cadmium contaminated soil by Indian mustard and spinach as influenced by phosphate fertilizer and mycorrhizae’ comprising of the pot culture experiment in the net house of Indian Institute of Soil Science (IISS), Bhopal and subsequent analyses in the laboratory of Soil Biology Division.

Location of experimental site

Experimental site is situated at Indian institute of soil science, Bhopal (MP) Latitude and Longitude (23°20'36.1"N 77°25'30.8"E), 485 m above mean sea level and has sub-humid tropical climate with a mean annual air temperature of 25°C and average annual rainfall of 1208 mm.

2.1. Materials
2.1.1. Soil
2.1.2. Treatment combination
2.1.3. Fertilizers
2.1.4. Seeds of Mustard and Spinach
2.1.5 Pots
2.2. Method
2.2.1. Greenhouse study
2.2.1. Pot culture experiments
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2.1. Materials

2.1.1 Soil

Cadmium contaminated Soil (0-15 cm depth) in bulk was collected from Patra-Nalah, Bhopal block of Bhopal district, Madhya Pradesh Latitude and Longitude location 23°20'36.1"N 77°25'30.8"E. The uncontaminated soil was also collected from the general farm Indian Institute of Soil Science, Bhopal. The both soils belong to vertisol *order*.

2.1.2. Treatment combination

Phosphates being used as treatments applied through AM and SSP are procured from provided Department of Soil Science and Agriculture Chemistry, R.A.K. College of Agriculture, Sehore, Madhya Pradesh, India.

2.1.3. Fertilizers

Urea, SSP, MOP were obtained from Department of Soil Science and Agriculture Chemistry, R.A.K. College of Agriculture, Sehore, Madhya Pradesh, India.

2.1.4. Seeds of Mustard and Spinach

Seeds of Indian Mustard (*Brassica spp*.) cv. Pusa Bold and spinach (*Spinacea oleracea*) cv. Pusa All green are popular cultivar in the same area from where soil sample was collected used as a test crops.

2.1.5. Pots

Plastic pots were used for growing are mustard and spinach seeds. The conical shaped pots of 6 kg capacity with holes at the bottom were used.

2.2 Methods

After collection of soil samples in bulk, these were sieved through 2 mm sieve before analysis of various physico-chemical properties. The pots were filled with six kilogram dry soil and used for greenhouse experiment. The uncontaminated soil was spiked @ 100 mg Cd kg^{-1} with cadmium nitrate [$Cd(NO_3)_2$] of solution and kept for one months before pot culture experiment.

2.2.1. Greenhouse study

The greenhouse study was conducted at IISS- Bhopal for growing mustard and spinach seeds from December 2014 to April, 2015.

2.2.1.1. Soil processing for pot culture study

The soil in bulk was air dried in shade and passed through 2 mm sieve and mixed thoroughly to attain uniformity.

2.2.1.2. Treatments and design

- **Soil: Two**

The following two soils were used for the study.

I. Naturally contaminated soil (NC) – This soil was collected from the contaminated site.

II. Cadmium spiked soil (Cd) – This uncontaminated soil was spiked with cadmium@100 mg kg^{-1} for two months before the initiation of study.

- **Source of phosphatic fertilizer: Three**

1. Control (without P)
2. Single superphosphate (SSP)
3. AM (Arbuscular mycorrhizae)

- **Growing cycle of mustard: One (Mustard) and Two (Spinach**)

1. Mustard 1st cycle (90 days)
2. Spinach two successive growing cycles (45 days and 90 days)

Treatment combination for this study

1 T1: Control (RDF=Recommended dose of fertilizer)
2 T2: RDF + SSP (Single super phosphate)
3 T3: RDF + AM (Arbuscular mycorrhizae)
4 T4: RDF + SSP + AM

Replication: Three (3)

Design of the experiment:-The experiment was laid out in a factorial completely randomized Design (CRD).

2.2.1.3. Spiking of Cadmium

For preparation of 100 ppm cadmium in spiked soil 1.4064 g cadmium nitrate [$Cd(NO_3)_2$] was dissolved in 1L of distilled water and this solution was applied to the 6 kg soil used for this study and was kept for one month before initiation of pot culture experiment.

2.2.1.4. P source addition through phosphatic fertilizer (SSP) and arbuscular micorhizza (AM)

The P sources through SSP and AM was applied in both soil of cadmium contaminated and spiked soil. About 4.12 g SSP applied and 12 g AM applied and mixed the treatment with soil by manually. (culture in powder form was measured in weight balance)

2.2.1.5. Pot culture study with mustard and spinach

The recommended dose of fertilizer for mustard 100:50:25 NPK was applied through urea, SSP and MOP (2.86 g urea, 4.12 g SSP, 0.548 g MOP respectively).For Spinach the recommend dose is 90:18:60 NPK was applied through urea, SSP and MOP (2.58 g urea, 1.48 g SSP, and 1.32 g MOP, respectively).

2.2.1.6. Sowing of mustard and spinach seeds

The variety of mustard (Pusa Bold) and spinach (All Green) was sown during the second week of December 2015. Before sowing the pots were filled with 6 kg soil and NPK recommended dose of fertilizer were for mustard and spinach added in the both naturally contaminated and cadmium spiked soil.

2.2.2. Sampling methods and analyses

2.2.2.1. Initial Soil samples

- **Analysis before initiation of the experimen**t

For obtaining information on the initial physicochemical characteristics of soils the sampling was done before initiation of the experiment and the samples were analyzed for the following parameters.

Table 2 Analysis of naturally contaminated and spiked soil sample before initiation of the experiment

Parameter	Cd Spiked soil	Natural cd contaminated soil	STDEV
Chemical parameter			
pH	7.78	6.88	0.64
EC (dSm^{-1})	0.28	0.23	0.03
Organic carbon (%)	0.44	0.86	0.30
CEC [cmol (P^+) kg $soil^{-1}$]	48	45	2.12
Nitrogen (kg ha^{-1})	83.6	62.7	14.78
Phosphorus (kg ha^{-1})	53.8	79.8	18.38
Potassium (kg ha^{-1})	317.7	452.9	95.56
Heavy metal			
Cd (ppm)	0.463	2.01	1.09
Pb (ppm)	1.12	3.3435	1.57
Cr (ppm)	0.524	1.842	0.93
Ni (ppm)	0.654	1.4975	0.60
Cu (ppm)	1.818	2.402	0.41
Zn (ppm)	2.15	0.823	0.94
Fe (ppm)	1.457	1.845	0.27
Mn (ppm)	0.684	1.457	0.55
Biological activity			

DHA (µg TPF g^{-1} soil $24h^{-1}$)	63.35	14.2505	34.72
FDA (*µg* fluorescein g^{-1})	9.181	4.926	3.01
SMBC	0.59	1.04	0.32
Soil respiration (mg CO_2-C g^{-1} soil d^{-1})	0.04	0.145	0.07
Microbial population			
Fungi (cfu)	3.33	5	1.18
Actinomycites (cfu)	7.67	7.17	0.35
Bacteria (cfu)	15	17	1.41
N-fixer (cfu)	20.67	27.835	5.07
P-solublizer (cfu)	CNF	CNF	---

*CNF - Colony not found

Estimation of Soil pH *(Jackson, 1973)*

Soil water extract was prepared by taking 20 g of soil and 40 ml of distilled water in 100 ml of beaker. The whole extract was then mixed with a glass rod. The pH meter was calibrated by immersing the electrodes in different buffer solution of pH 4.0, 7.0 and 9.2. Dipped the electrodes into beaker containing the soil extract and noted the reading displayed by the pH meter.

Estimation of Soil EC *(Jackson, 1973)*

Soil water extract was prepared by 20 g of soil sample and 40 ml distilled water (i.e. 1:2 ratio) in 100 ml beaker. The extract mixed with a glass rod. The electrical conductivity meter was adjusted at temperature 25°C and calibrated with the standard solution 0.01N KCl solution conductivity 1.41 (dSm^{-1} at 25°C). Before proceeding for the samples, washed the conductivity cell for avoiding error. The conductivity cell washed after each determination and wiped out excess water with tissue paper.

Estimation of Moisture content *(Gravimetric method)*

The moisture content of the soil was measured gravimetrically. The moisture percentage was calculated by using following formula–

Moisture % = (Wt of wet soil – Wt of dry soil / wt of dry soil) X 100

Estimation of Organic carbon (%), *(Walkley and Black Method, 1934)*

1 g soil sample was taken in 500 ml conical flask and 10 ml of 1N $K_2Cr_2O_7$ (Potassium dichromate) and 20 ml concentrated H_2SO_4 (Sulphuric acid) and mixed by gentle shaking. The flask is allowed to stand for 30 minutes and thereafter 200ml of distilled water and 10 ml of ortho-phosphoric acid was added. Before titration with

0.5 N ferrous ammonium sulphate (FAS) 1 ml of diphenylamine indicator was added where at end it apperars to green from blue violet.

Estimation of Available N, Modified alkaline Permanganate method, *(Subbiah and Asija, 1956)*

20 g of soil sample was placed in 800 ml Kjeldahl flask. Moisten the soil with about 10 ml of distilled water, washed down the soil, if any, adhered to the neck of the flask. Then 100 ml of 0.32% of $KMnO_4$ solution was added followed by addition of few glass beads or broken pieces of glass rod to avoid bumping. Then 2-3 ml of paraffin liquid was added to avoid contact with upper part of the neck of the flask. Then measured about 20 ml of 2% boric acid containing mixed indicator in a 250 ml conical flask and placed into the receiver tube. Run tap water through the condenser. Added 100 ml of 2.5% NaOH solution and immediately attached to the rubber stopper fitted in the alkali trap. Switch the heaters on and continue distillation until about 100 ml of distillate was collected. The conical flask containing distillate was removed and then switch of the heater to avoid back suction. Titrate, the distillate was titrated against 0.02M H_2SO_4 taken in burette until pink colour starts appearing. A blank without soil sample and carefully remove the Kjeldahl flask after cooling and drain the contents in the sink.

Estimation of Total Nitrogen, Kjeldahl Method, *(Bremner, 1965)*

Total N was estimated by Kjeldahl digestion-distillation method where the evolved ammonia was collected in 4% boric acid and titrated against standard 0.02 N sulphuric acid (Bremner, 1965).

Estimation of available phosphorus, Olsen's method, *(Olsen, et al, 1954)*

2.5 g of processed soil sample was shaken with 50 ml of 0.5 M $NaHCO_3$ extractant keeping the pH to 8.5 (Olsen *et al.* 1954) together with 1 g of Darco G-60 (free from soluble phosphorus) for 30 minutes in a 250 ml conical flask on mechanical shakers and then filtered through filter paper.

Development of colour

5 ml of colour less filtrate was taken in 25 ml volumetric flask for determination and then 5 ml of 1.5 per cent ammonium molybdate solution was added. The contents were diluted to about 20 ml with distilled water shaken and then 1 ml of fresh working solution (stannous chloride) solution was added to develop blue colour and diluted to the mark and shaken thoroughly. The colour intensity was measured in a spectrophotometer within 10 minutes at 660 nm wavelength after setting the instrument to zero with blank prepared similarly but without soil as described by

Jackson (1973). The amount of available phosphorus was calculated and the results were expressed in kg P/ ha by using standard curves.

Estimation of available potassium (K_2O kg^{-1}), Neutral ammonium acetate method, *(Toth and Prince, 1949)*

Preparation of the Standard Curve: Set up the flame photometer by atomizing 0 and 20 µg K/ml solutions alternatively to 0 and 100 reading. Atomize intermediate worked standard solutions and record the readings. Plot these readings against the respective potassium contents and connect the points with a straight line to obtain a standard curve.

Extraction: Added 25 ml of the ammonium acetate extractant to conical flask fixed in a wooden rack containing 5 g soil sample. Shake for 5 minutes and filtered. After this process determined potash in the filtrate with the flame photometer.

Estimation of sulphur, Turbidimeteric method, *(Chesnin and Yien, 1951)*

Sulphar was extracted with 0.15% $CaCl_2$ and the soluble sulphate measured turbidimatrically on a spectrophotometer used blue filter at 340 mm. 5 g soil was transferred into an 150 ml Erlenmeyer flask. 25 ml of 0.15% $CaCl_2$ was added to it shaken for 30 minutes was done on a reciprocation shaker, then filtration of the suspension was done through whatman No.42 filter paper.

Development of turbidity

Pipetted out 10 ml extract in a 150 ml Erlenmeyer flask and to this 20 ml of water was added thus brought the volume to solution and 0.2 g of $BaCl_2$ crystals was added into the flask. The flask was shaken for 1 minute each at constant rate. After 3 minutes turbidity was measured in a colorimeter using blue filter. The amount of sulphur was calculated and the results were expressed in mg kg^{-1} by used standard curve.

Estimation of Cation Exchange Capacity (CEC)

Weighed 5 g soil and transferred the sample to a 50 ml centrifuge tube and then transfer 25 ml of 1.0M sodium acetate solution was added to the tube, stopped and shaken in a mechanical shaker for 5 minutes. Then centrifuged at 2000 rpm for 5 minutes or until the supernatant liquid is clear and decanted the liquid completely and repeated the extraction three more times. Discard the decants. Repeated steps 2 – 4 with ethanol or isopropyl alcohol until the EC of the decant reads less than 40 mS/cm (usually it takes 4 to 5 washings). To displace the adsorbed Na, repeat steps 2 – 4 using the ammonium acetate solution. Collected the decant in 100 ml volumetric flask fitted with a funnel and filter paper. Make up to volume with ammonium acetate solution and determine sodium concentration by flame photometry, prepare a series of Na standard solutions in the range of 0 – 10 meq/ litre of Na. Prepared a standard curve by plotting sodium concentration on x-axis and flame-photometric readings on y-axis. Unknown sample extract is fed on the flame-photometer and the reading is taken, corresponded to which the concentration of sodium is readed from the standard curve. For better results, add LiCl in each standard to yield a final concentration of about 5 meq/litre of LiCl.

Estimation of Heavy metals (Cd, Zn, Cu, Pb, Ni), DTPA extractable metal *(Lindsay and Norvell, 1978)*

10 g of soil sample in 100 ml conical flask and includes 20 ml of the DTPA extractant and shaken for 2 hours on a mechanical shaker. Filtered through whatman No. 42 filter paper, discarded first few drops. For quick filtration, whatman No. 1 filter paper can also be used if the filtrate is clear. This filtrate use for Cd, Zn, Cu, Pb, Ni measurement of AAS. Feed the standard working solutions and prepare a standard curve by plotted AAS readings against Cd, Zn, Cu, Pb, Ni concentrations.

Biological analysis

Estimation of Dehydrogenase enzyme activity, *(Klein et al, 1971)*

Dehydrogenase activity was estimated by monitoring the rate of production of tri-phenyl formazon (TPF) from tri-phenyl tetrazolium chloride (TTC), which was used as an electron acceptor. The method of Klein *et al.* (1971) was followed for the assay of dehydrogenase activity as outlined below.

1 g of air-dried soil was placed in 15 ml screw capped tube. To this 0.2 ml of 3% TTC and 0.5 ml of 1% glucose were added and ensured to make the system anaerobic, the tubes were incubated at 28^{o}C for a period of 24 hours. After incubation 10 ml of methanol was added and shaken for exactly one minute. It was allowed to stand in dark for six hours. The colour intensity developed, was measured at 485 nm (blue filter). From the standard curve, drawn in the range of 0.004 to 0.4 mg TPF per 10 ml of methanol, the TPF produced in the samples were computed. Dehydrogenase activity was expressed as TPF formed per g soil for 24 hours on oven dry weight basis.

Estimation of Soil Respiration, Alkali trap method *(Ausmus and O'Neill, 1978)*

It was determined moisture content of the soil sample gravimetrically. Take 100 g soil oven dry in 500 ml conical flask. Add was water to bring its moisture content to field capacity, unless you intend to study the effect of moisture content. Take 20 ml of 0.5 N NaOH in test tubes. Hang the tubes with the help of thread inside the conical flask without touched the soil. Make the flasks air tight by rubber stopper keep the flask in an incubator at 28^{o}C. After different specific time intervals (e.g., 1-day, 2-days, 7-days etc.) of incubation, take out the flask from the incubator. Transferred the 0.5 N NaOH solution from the test tubes to the beakers. Give several washed of the complete transfer. Add few drops were of saturated $BaCl_2$ with few drops of Phenolphthalein indicator. Titrate with standard 0.5 N H_2SO_4 slowly until the pink colour just disappears. Approach the end point with caution and recorded the exact amount of acid required. Repeat the above steps 3 to 9 if measurement has to be continued for longer duration.

Estimation of Fuorescein di-acetate (FDA), *(Adam and Duncan, 2001)*

2 g of soil (fresh weight, sieved 2 mm) was placed in a 50 ml conical ask and 15 ml of 60 mM potassium phosphate buffer pH 7.6 added. Stock solution (0.2 ml 1000 mg FDA ml21) was added to start the reaction. Blanks were prepared without the addition of the FDA substrate along with a suitable number of sample replicates. The fasks were stoppered and the contents shaken by hand. The fasks were then placed in an orbital incubator (Gallenkamp Orbital Incubator, 100 rev min21) at 30C for 20 min. The following steps involving chloroform/methanol were carried out in a fume cupboard. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was added immediately to terminate the reaction. Stoppers were replaced on the fasks and the contents shaken thoroughly by hand. The contents of the conical asks were then transferred to 50 ml centrifuge tubes and centrifuged at 2000 rev min21 for approximately 3 min (MSE Scientic Instruments, Coolspin 2 centrifuge). The supernatant from each sample was then filtered (Whatman, No 2) into 50 ml conical fasks and the ltrates measured at 490 nm on a spectrophotometer (Hitachi U-1100 spectrophotometer).

The concentration of fuorescein released during the assay was calculated using the calibration graph produced from 0 to 5 mg fuorescein ml21 standards which were prepared from a 20 mg fuorescein ml 21 standard solution. The 0 mg ml21 fuorescein standard was used to zero the spectrophotometer before each set of blanks and samples were read.

Estimation of Soil microbial biomass carbon, *(Voroenyet at. 1993)*

The microbial biomass carbon in the soil was estimated by fumigation extraction method. Moist sample was taken in duplicate (to give approximately 10 g oven-dry weight) in 50 ml glass beakers. One set was kept inside a vacuum desiccator and fumigated with fresh ethanol-free chloroform for 24 hours and the excess chloroform was removed by repeated back suction at the end of fumigation (Jenkinson and Powlson, 1976). The fumigated and non-fumigated soils were extracted with 25 ml of 0.5*M* K_2SO_4, solution and the extract was digested in presence of potassium persulphate ($K_2S_2O_8$) and 0.025*M* H_2SO_4 in a digestion block at 120^0C for 2 hours. The amount of CO_2-C that evolved was estimated by following the modified procedure of Jenkinson *et al*. (1979) and Tate *et al.* (1988). The amount of the MBC calculated as fallows:

$$\text{Microbial biomass carbon} = (OC_F\text{-}OC_{UF}) / K_{EC}$$

where, OC_F and OC_{UF} are organic carbon extracted from fumigated and unfumigated soil respectively (expressed on oven dry basis), and K_{EC} is the efficiency of extraction. A K_{EC} of 0.25 as a general value for microbial extraction efficiency was used for calculation.

Estimation of Soil microbial population, **(Fungi, Bacteria, Actinomycetes),** *(Chhonkar et al, 2006)*

Media Preparation:-

1. Nutrient Agar Media (for Bacteria)

Nacl	5 g
Peptone	5 g
Beaf extract	3 g
Agar–agar	18 g
Volume to make distilled water	1 L
pH	7

2. Rose Bengal red media (for Fungi)

Dextrose	10.0 g
Peptone	5.0 g
Potassium di- hydrogen phosphate	1.0 g
Magnesium sulphateheptahydrate	0.5 g
Agar–agar	20.0 g
Streptomycin (Antibiotic)	traces* (Use just before plating)
Volume to make distilled water	1 L

To be added after autoclaving and just before plating. Final concentration of streptomycin should be about 30 ppm.

3. Kenknight and Munaier’s media (for Actinomyceties)

Dextrose	1.0 g
Potassium di-hydrogen phosphate	0.1 g
Sodium nitrate	0.1 g
Potassium chloride	0.1 g
Magnesium sulphateheptahydrate	0.1 g
Agar-agar	15.0 g
Volume to make distilled water	1L

Procedure:-

Separated soil closely adhered to the plant roots after gentle tappen / shaken. This was constituted rhizosphere soil sample. The other soil sample from non-cropped area was non–rhizosphere sample. Prepared serial dilutions for plated of the soil within the laminar flow assembly and added 10 g soil sample in 95 ml sterile water blank in 500 ml conical flask. Replaced cotton plug with surface sterilized rubber stopper. Shacked thoroughly too dispersed soil by given vertical strokes. The shaken was uniform for all soil samples. Transfer 1 ml soil suspension was obtained in the previous step into 9 ml sterile water blank in the test tubes. Shaken was uniformly between palms of your hands to provided horizontal shaken. Continue the series in similar manner to get up to 10^{-6} dilution level. Mark the dilutions properly on the test tubes. Transfer 1 ml of requited dilution (10^{-4} for fungi and actinomycetes and 10^{-5} to 10^{-6} for bacteria) into sterile petri plates. Pout the required selective media (at approx 48^{o}C) for specific organisms uniformly into plates. To rate clockwise and anti-clockwise to mix soil suspension with medium. Make the rotation uniform for all samples. Allow the medium to solidify. Make details on the lid. Incubate plates in inverted position at 28^{o}C in the incubator for 48 hrs for fungi; 4 days for bacteria and one week for actinomycetes. Take at least four replications for each sample. Examine plates for the colonies developed after the required incubation period. Shatch colonies and note pigmentation if any. Count the number of colonies. Discard plates shown large spreaders and mold colonies. Number of colonies multiplied by the respective dilution factor was given number of viable cells g^{-1} soil. Examine most dominant colonies under microscope. Calculate standard deviation and standard error of mean. Statistically compare the number of organisms in the rhizosphere soil samples.

Estimation of P- solublizer, *(Pikovskaya's method, 1948)*

Media Preparation:-

Pkovaskaya media for P- solubilising bacteria

Glucose	10 g
Yeast extract	0.5 g
$(NH_4)_2SO_4$	0.5 g
KCL	0.2 g
NaCl	0.2 g
$MgSO_4.7H_2O$	0.1 g
$FeSO_4.7H_2O$	Trace
$MgSO_47H_2O$	Trace
$Ca_3(PO_4)_2$	5.0 g
Agar	18.0 g
pH	7.0 – 7.2

Procedure :-

Suitable dilutions (10^{-4}) of serially diluted rhizosphere soil suspension were poured plated on Pikovskaya's Agar [Glucose – 1 g, $Ca(PO_4)$- 5 g, $(NH_4)_2.SO_4$- 0.5 g, KCL – 0.2 g, $MgSO_4$ – 0.1 g, $MnSO_4$ – traces, FeSO4 – trace, Yeast extract – 0.5 g ,Agar – 15 g, Distilled Water – 1 L, pH- 7.0 – 7.2] and the plates were incubated at 30^oC for 48 – 96 hours. Phosphate solubilization is indicated by the formation of a solubilization or a clear zone around the bacterial colonies. Single bacterial colonies having clear solubilization zones were isolated separately on to fresh Pikovskaya's agar plates, incubated at 30^oC

Estimation of N – Fixer, *(Okon et al,1977)*

Media Preparation:-

Jensen's agar media for living N – fixers

Sucrose	20.0 g
K_2HPO_4	1.0 g
$MgSO_4$	0.5 g
NaCl	0.5 g
Na_2MoO_4	0.001 g
$FeSO_4$	0.01 g
$CaCO_3$	2.0 g
Agar	18.0 g
pH	7.0 – 7.2

Procedure :-

Weighed 10 g of representative soil sample (Rhizosphere). Prepared serial dilutions up to 10^{-3} and pipetted out 1.0 ml soil suspension (as an inoculum) from 10^{-1} to 10^{-3} dilutions in sterilized petridishes separately. Prepare melted Jensen's Agar media (45 – 50^{o}C) in the plates aseptically. After solidification of the media, keep the plates for incubation at 28 – 30^{o}C for 24 – 48 hrs in B.O.D. incubator. After incubation period, colonies shown light brown to black pigement of Jensen's media should be selected for isolation.

Estimation of Glomalin extraction, *(Wright et al., 1996, Wright &Upadhyaya, 1996 & Wright & Upadhyaya, 1998)*

Procedure :-

1.0 g of soil in centrifuge tube with 8 ml 50mM sodium citrate (smaller samples may be extracted as long as this ratio is adhered to). Autoclave for 60-90 min at 121^{0}C (A 30 min extraction may be used on soil to remove the fresh protein and compare with the 60 min total extraction. However, the 60 min extraction is used for pot cultures as a total protein assay). Centrifuge at 5000 x g for 15 min immediately after extraction (centrifugation is just pellet the soil particles and may be conducted at any speed from 3000-10000 xg). Remove the supernatant containing the protein and store* at 4^{0}C. Measure total volume of extract with a graduated cylinder. Transfer 1 ml extract in test tube and add 1 ml Bradford dye solution than colure change this solution. Reading note by spectrophotometer in 595 nm.

2.2.2.2. Collection and processing of plant and soil samples

Plant samples

Mustard

At maturity (95 DAS) of mustard plant, the whole plant was taken from the pot and the recorded the fresh weight of whole plant. The roots were carefully separated from the adjoining soil and cleaning under a gently through tap water. The roots were dipped in deionized water and were subsequently dried in blotting paper to remove excess water. The straw, grain and roots samples were dried in shade followed by oven dried at 70^{0}C until constant weight was achieved and plant sample divided in stem, root, and pod and collected the grain from pod of mustard for various nutritional parameters as well as Cd content.

Spinach

After 45-47 days maturity the whole spinach plant with soil was taken out of the pot and the recorded fresh weight of whole plant. The leaves were carefully separated from the adjoining soil cleaning under a gently through tap water. The roots were dipped in deionized water and were subsequently dried in blotting paper to remove excess water. The straw, samples were dried in shade followed by drying in a oven at 70^0 C until constant weight was achieved. The biomass yield was recorded and the samples were ground for analysis of cadmium.

2nd cycle of spinach after the harvesting 40-45 days maturity the whole spinach plant with soil was taken out of the pot and the recorded fresh weight of whole plant. The leaves and root were carefully separated from the adjoining soil cleaning under a gently through tap water. The roots were dipped in deionized water and were subsequently dried in blotting paper to remove excess water. The straw samples were dried in shade followed by drying in a oven at 70^0C until constant weight was achieved. The biomass yield was recorded and the samples were ground for analysis of cadmium.

- **Soil Samples**

Mustard and Spinach crop experiment

During the separation of the roots from the soil, the soils adhering strongly to the root mass were collected as rhizosphere soil. Fresh soil samples were sieved through 2 mm sieve. Part of the fresh soil sample (2 mm sieved) was stored in a freezer at 4^0C for the determination of microbial activity and other biological activities. The rest of the soil sample was dried in the shade for determination of available, total and various cadmium fractions in soil. The soils of the initial were also analyzed for above parameters.

Collect of soil sample in every pot to polythein bag for chemical analysis and this soil sample are dry in atmosphereic condition and sieve 2 mm sieved.

Mustard and Spinach experiment

The soil from each pot in mustard and spinach experiment was also collected in the same way as done in harvest of mustard and spinach experiment.

2.2.2.3. Methods of soil and plant analysis

Soil samples collected from each pot without crops of experiment, after completion of each growing cycle of crops and after harvesting of mustard and spinach were used to determine the following parameters.

Estimation of Nitrogen in straw and grain, Micro Kjeldhal method, *(Chapman and Pratt, 1982)*

Nitrogen content in straw and grain sample was determined by Modified Kjeldahl Method as per procedure outlined (Gupta, 2007). In a digestion tube 0.5 g of powdered plant straw was taken and 10 ml of diacid solution (9:4, HNO_3:$HClO_4$) was added and kept for overnight then 10g of sulphate mixture [(20 parts K_2SO_4) + 1 part catalyst mixture (20 parts $CuSO_4$ + 1 part selenium powder)] was added and heating was done in a digestion chamber till a clear colour less solution appears, then cooled and filtered through Whatman No. 42 filter paper in a 50 ml volumetric flask and made up to the volume with distilled water.

Ten ml of 4% boric acid solution containing bromocresol green and methyl red indicator was taken in a conical flask, outlet of distillation apparatus was dipped into boric acid solution. Five ml of the aliquot was taken and transferred to distillation flask of micro-kjeldahl distillation apparatus and 10 ml of 40 % NaOH solution was added. After completion of distillation, the boric acid was titrated against 0.02 N H_2SO_4. Blank was also run. And N content was calculated by formula.

Digestion for P, K, in straw and grain

Digestion process :-

0.5 g dried and powdered plant sample (20 mesh) was taken in a 50 ml digestion tube and 10 ml di-acid mixture (9:4 v/v HNO_3: $HClO_4$) was added to it and was kept overnight. It was then digested on a block digester till a colorless solution was obtained. The volume of acid was reduced till the flask contained only moist residue. The flask was cooled and 100 mL of distilled water was added. The solution was filtered into a 100 mL volumetric flask and diluted up to mark.

Estimation of phosphorus in straw and grain, Vanadomolydo yellow colour method, *(Koeing and Johnson, 1942)*

The phosphorus content in mustard straw, grain and spinach straw were determined as per procedure outlined (Gupta, 2007). Five ml of plant digest was taken in a 50 ml volumetric flask and 10 ml of vanadomolybdate reagent was added in it. The volume was made up to the mark with distilled water, it was shack in thoroughly. Then the absorption of solution was recorded after 30 min at 420 nm on spectrophotometer using blue filter. First standard reading was taken followed by sample reading. Phosphorus content in straw and grain was calculated using standard curve and expressed as total P (%).

Estimation of potassium in straw and grain, Di-acid digestion method by Flame photometer, *(AOAC, 1984)*

Potassium content of straw and grain was determined by Flame Photometer method (Jackson, 1973). The digested extract was used directly for flame photometer determination of potassium. K content was calculated using the standard curve and expressed as total K

(%).

Estimation of Sulphur in straw and grain, Turbidimetric Method, *(AOAC, 1984)*

Digestion process :-

0.5 g dried and powdered plant sample (20 mesh) was taken in a 50 ml digestion tube and 10 ml di-acid mixture (9:4 ratio of HNO_3: $HClO_4$) was added to it and was kept overnight. It was then digested on a block digester till a colorless solution was obtained. The volume of acid was reduced till the flask contained only moist residue. The flask was cooled and 100 mL of distilled water was added. The solution was filtered into a 100 mL volumetric flask and diluted up to mark.

Procedure :-

10 ml of digested plant aliquat was taken in 50 ml volumetric flask and then 1 g Barrium chlorid powder was added and make up the volume 50 ml with distilled water. It was then Shaken 10 min. and reading was noted by spectrophotometer at 420nm.

Estimation of Cadmium (Cd) in straw and grain, *Intwongse and Dean (2006)*

0.5 g dried and powdered plant sample (20 mesh) was taken in a 50 ml digestion tube and add 5 ml HNO_3 was added to kept for overnight for pre digestion. After 24 hours 10 ml di-acid mixture (9:4 v/v HNO_3: $HClO_4$) was added to it and was took at hot plate at 70-80^oC for 1 hours and 1 hours after temperature increase to 120-130^oC for 4-5 hours. After some time colour changed from brown to white. The volume of acid was reduced till the flask contained only moist residue. The flask was cooled and 100 mL of distilled water was added. The solution was filtered into a 100 mL volumetric flask and diluted up to mark and reading was noted by ICP.

2.2.3. Statistical analysis :-

Data obtained was statistically analyzed following standard statistical methods (Gomez and Gomez, 1984). Factorial Completely Randomized Design (CRD) was used for evaluating the main effects as well as interaction effects of the two factors. Two factor CRD was done using the computer based statistical progme MSTAT-C.

CHAPTER – IV

RESULTS

In these chapter results of the pot culture experiment entitled **"Phytoremediation of cadmium contaminated soil by Indian mustard and spinach as influenced by phosphate fertilizer and mycorrhizae"** conducted during 2014-15 were presented. Data recorded on various observations during the course of study were tabulated and statistically analysed, and presented in figures and diagrams. This investigation was primarily undertaken to find out the effects of phytoremediation on cadmium contaminated soil with the application of phasphatic fertilizer and mycorrhizae. In this study we observed the effect of phytoremediation with respect to different treatments on growth parameters and nutrient uptake, heavy metal accumulation by Indian mustard and spinach as well as effect on soil biological parameters. The results are furnished in this chapter in the form of the tables and illustrated through the figures wherever necessary under appropriate headings.

Table 1.1 Effect of phytoremediation on N content (%) in mustard shoot under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.00	0.81	0.90
T2	**RDF+SSP**	1.11	1.14	1.13
T3	**RDF+AM**	1.19	1.15	1.17
T4	**RDF+SSP+AM**	1.38	1.14	1.26
Mean		1.17	1.06	
		SEm	**CD at % level**	
Treatment (T)		0.039	0.117	**SIG**
Stage (S)		0.028	0.083	**SIG**
Interaction (TxS)		0.048	0.143	**NS**

The effect of treatments and stage of plant growth had significant effects on N accumulation in the mustard plant (Table, Fig. 1.1 and Appendix 1.1). The T4 i.e. (RDF+AM+SSP) was found most prominent effect N accumulation in the mustard plant (1.26%) whereas T1 (RDF) was found in the least effect on N content (0.90%) in the plant. In growth stages of the crops the 45 DAS (1.17%) the plant had much higher N accumulation than the 90 DAS (1.06%). There was no significant role which the interaction of growing stage and treatment in the N accumulation of in mustard plant.

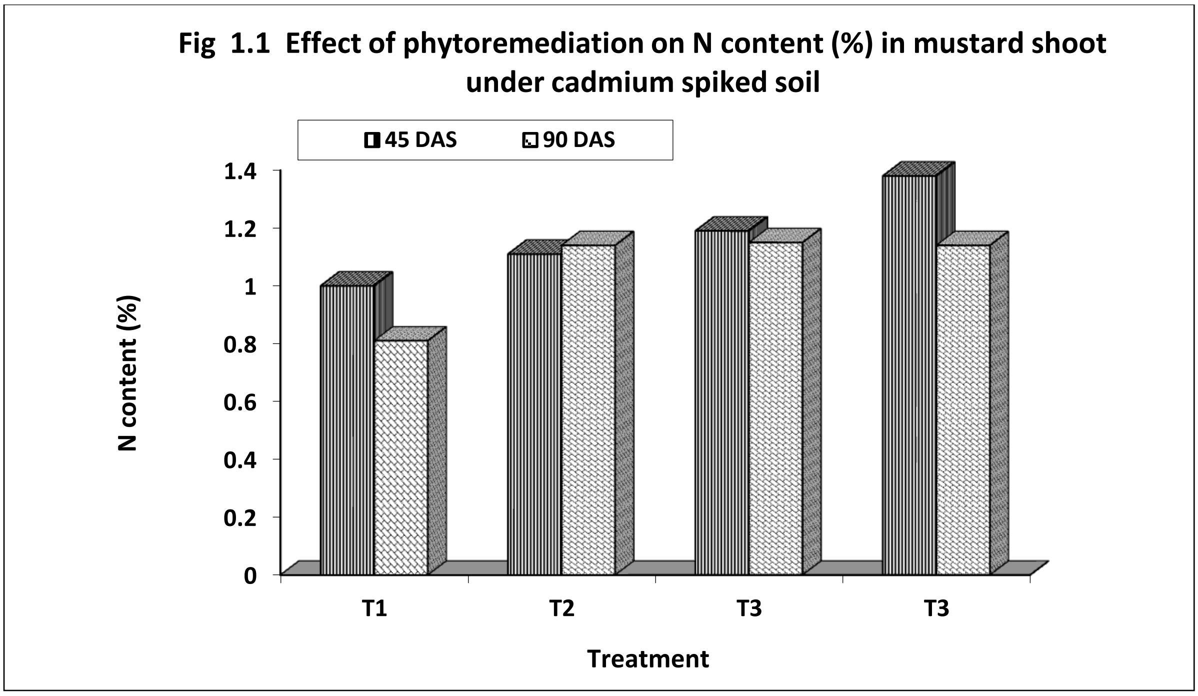


Fig 1.1 Effect of phytoremediation on N content (%) in mustard shoot under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

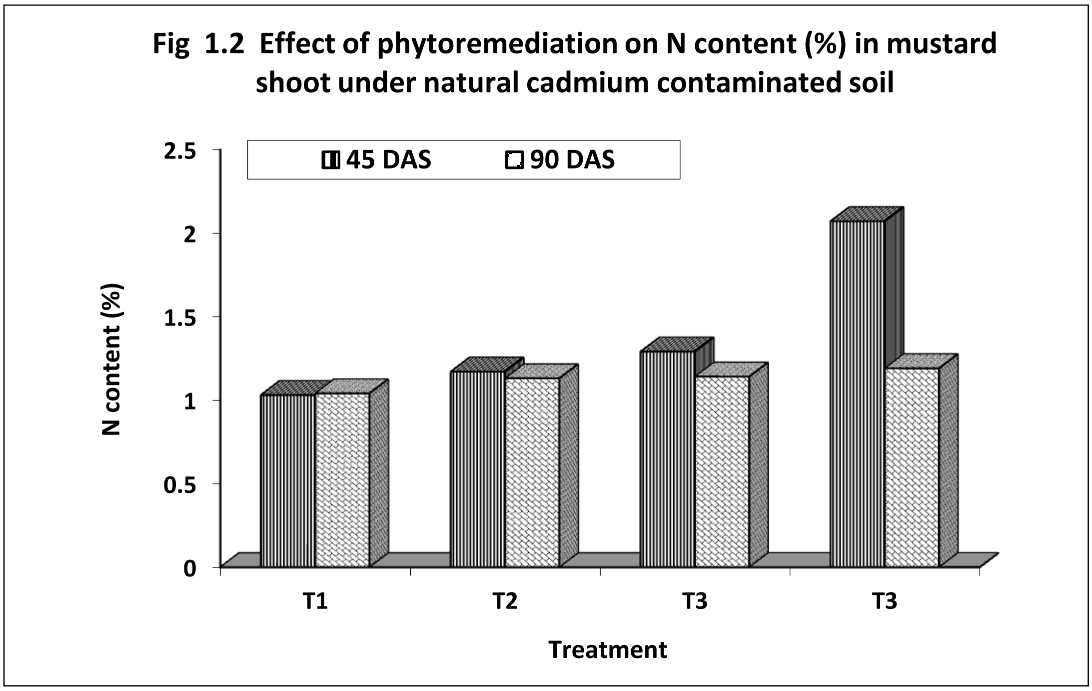


Fig 1.2 Effect of phytoremediation on N content (%) in mustard shoot under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate **T1 =** Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table1.2 Effect of phytoremediation on N content (%) in mustard shoot under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.03	1.04	1.03
T2	**RDF+SSP**	1.17	1.13	1.15
T3	**RDF+AM**	1.29	1.14	1.22
T4	**RDF+SSP+AM**	2.07	1.19	1.63
Mean		1.39	1.13	
		SEm	**CD at % level**	
Treatment (T)		0.112	0.337	**SIG**
Stage (S)		0.079	0.238	**SIG**
Interaction (TXS)		0.138	0.413	**SIG**

The effects of treatments and stage of plant growth had significant effect on N accumulation in the mustard plant (Table, Fig 1.2 and Appendix 1.2). The T4 (RDF+AM+SSP) was found most prominent effect on N accumulation in the plant (1.63%) whereas T1 (RDF) was found in the least content of N (1.03%) in the plant. In growth stages of the crops, the 45 DAS (1.39%) the plant had much the higher N accumulation than the 90 DAS (1.13%). In this study the interaction between growing stage and treatments was significant role on mustard plant N accumulation in naturally contaminated soil. Among the interaction T4 in 45 DAS was observed superior (2.07%) than other treatment combination with growing stage on mustard plant the N accumulation.

Table 1.3 Effect of phytoremediation on N content (%) in mustard root under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.14	0.34	0.24
T2	**RDF+SSP**	0.28	0.45	0.36
T3	**RDF+AM**	0.42	0.39	0.41
T4	**RDF+SSP+AM**	0.61	0.68	0.64
Mean		0.36	0.47	
		SEm±	**CD at 5% level**	
Treatment (T)		0.033	0.100	**SIG**
Stage (S)		0.024	0.071	**SIG**
Interaction (TxS)		0.041	0.123	**NS**

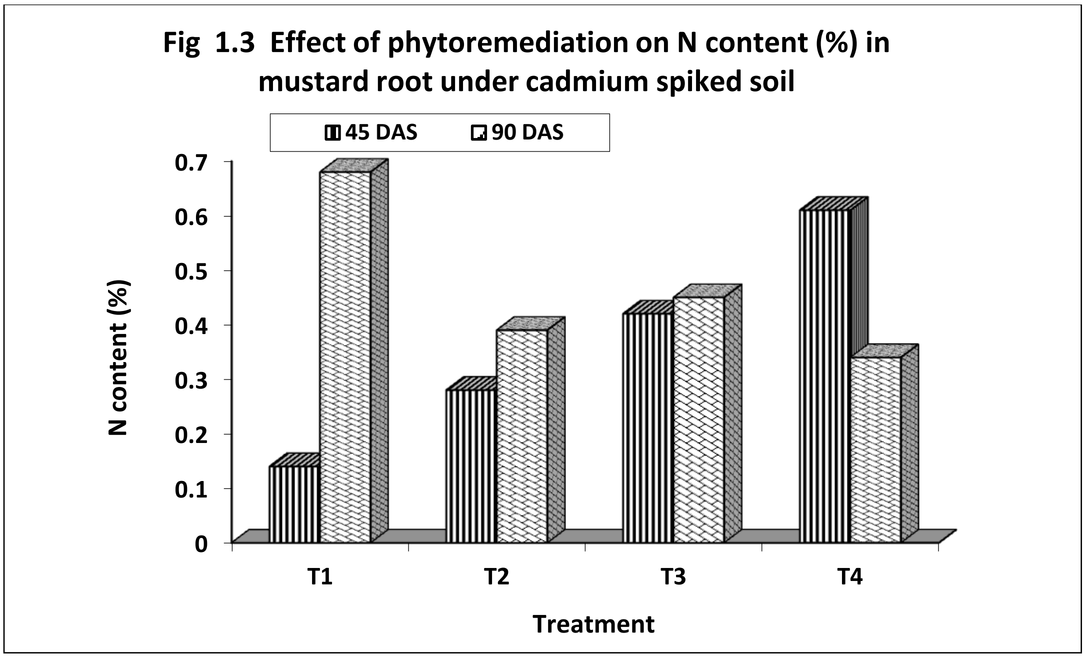


Fig 1.3 Effect of phytoremediation on N content (%) in mustard root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

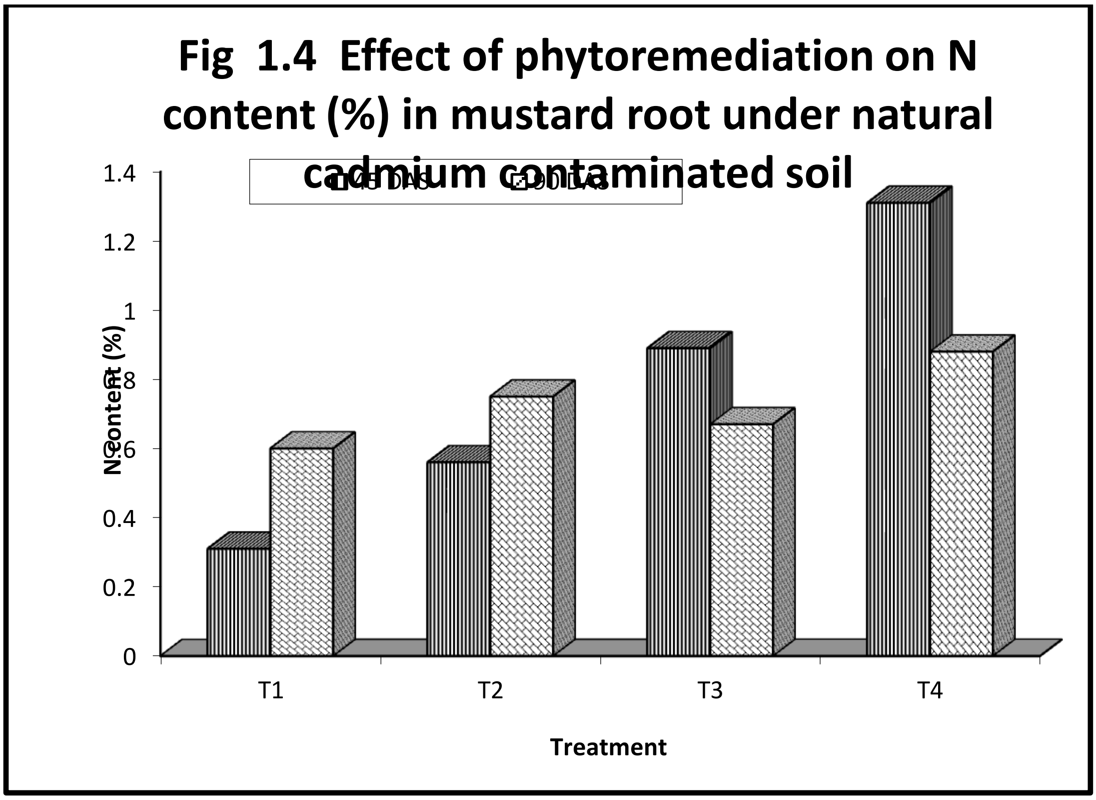


Fig 1.4 Effect of phytoremediation on N content (%) in mustard root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant role on N accumulated spiked soil in the mustard root (Table, Fig 1.3 and Appendix 1.3). The treatment T4 (RDF+AM+SSP) was found most prominent effect N accumulation in the mustard root (0.64%) where T1 (RDF) was observed in the least content (0.24%) in the plant. The stages of the crop growth had significant effect of N accumulation on the mustard root. In 90 DAS (0.47%) the plant had much the higher N accumulation than the 45 DAS (0.36%). There was no significant role in the interaction of growing stage and treatment in the N accumulation of in mustard root.

Table 1.4 Effect of phytoremediation on N content (%) in mustard root under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.31	0.60	0.45
T2	**RDF+SSP**	0.56	0.75	0.65
T3	**RDF+AM**	0.89	0.67	0.78
T4	**RDF+SSP+AM**	1.31	0.88	1.09
Mean		0.31	0.60	
		SEm±	**CD at 5% level**	
Treatment (T)		0.074	0.222	**SIG**
Stage (S)		0.052	0.157	**NS**
Interaction (TxS)		0.091	0.271	**SIG**

In this study the interaction between growing stage and treatment had significant role on N accumulation in naturally Cd contaminated. With respect to interaction effect, the growth stages of the crops the T4 treatment in 45 DAS (1.31%) was found superior than other treatment combination for N content in mustard root. The effects of treatment had significant effect on N accumulation in the mustard root (Table, Fig 1.4 and Appendix 1.4). The T4 (RDF+AM+SSP) was observed most prominent effect N accumulation in the root (1.09%) whereas T1 (RDF) was observed in the least content (0.45%) in the plant. The stages of the crop growth had non-significant effect of N accumulation on the root. In the growth stage of crops, 90

DAS (0.60%) the root had much the higher N accumulation than the 45 DAS (0.31%).

Table 1.5 Effect of phytoremediation on N content (%) in mustard grain under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.21	2.84	2.03
T2	**RDF+SSP**	1.77	3.38	2.58
T3	**RDF+AM**	1.96	3.62	2.79
T4	**RDF+SSP+AM**	2.10	4.71	3.40
Mean		1.76	3.64	
		SEm±	**CD at 5% level**	
Treatment (T)		0.481	1.441	**NS**
Stage (S)		0.340	1.019	**SIG**
Interaction (TxS)		0.588	1.764	**NS**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S, and Cd) content in the mustard grain.

The effects of treatment had non-significant effect on N accumulation in the mustard grain (Table, Fig 1.5 and Appendix 1.5). The stages of the crop growth had significant effect of N accumulation on the mustard grain. In the growth stages of crops in 90 DAS (3.64%) the plant had much the higher N accumulation than the 75 DAS (1.76%). There was no significant role which the interaction of growing stage and treatment in the N accumulation of in mustard root.

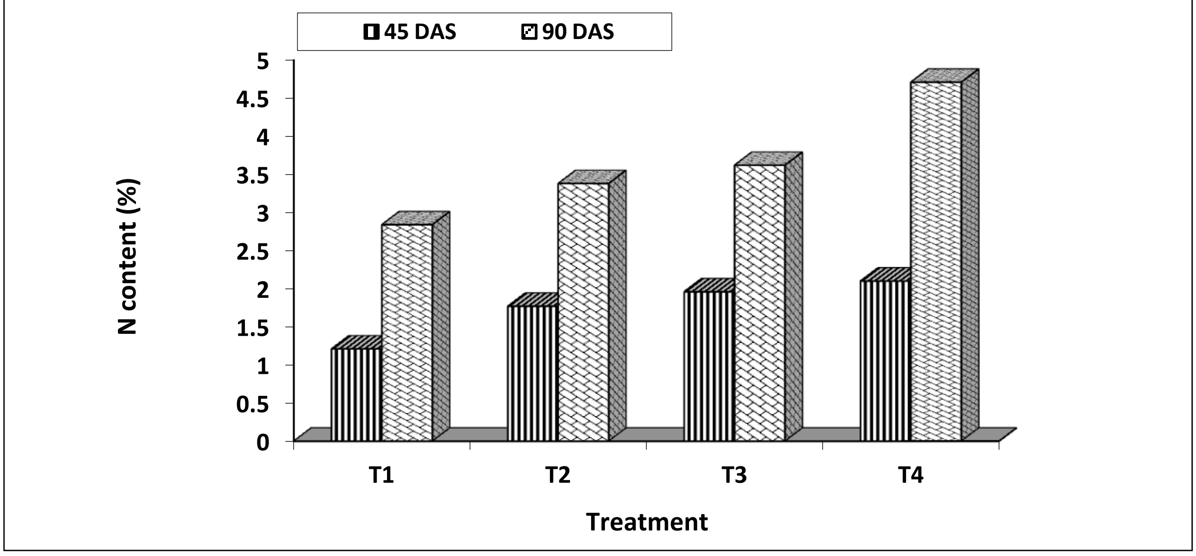
Table1.6 Effect of phytoremediation on N content (%) in mustard grain under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.82	3.44	2.63
T2	**RDF+SSP**	1.96	4.02	2.99
T3	**RDF+AM**	2.19	4.20	3.20
T4	**RDF+SSP+AM**	2.66	4.58	3.62
Mean		2.16	4.06	
		SEm±	**CD at 5% level**	
Treatment (T)		0.477	1.429	**NS**
Stage (S)		0.337	1.010	**SIG**
Interaction (TxS)		0.584	1.750	**NS**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S and Cd) content in the mustard grain.

The effects of treatment had non-significant effect on N accumulation in the mustard grain (Table, Fig 1.6 and Appendix 1.6). The stages of the crop growth had

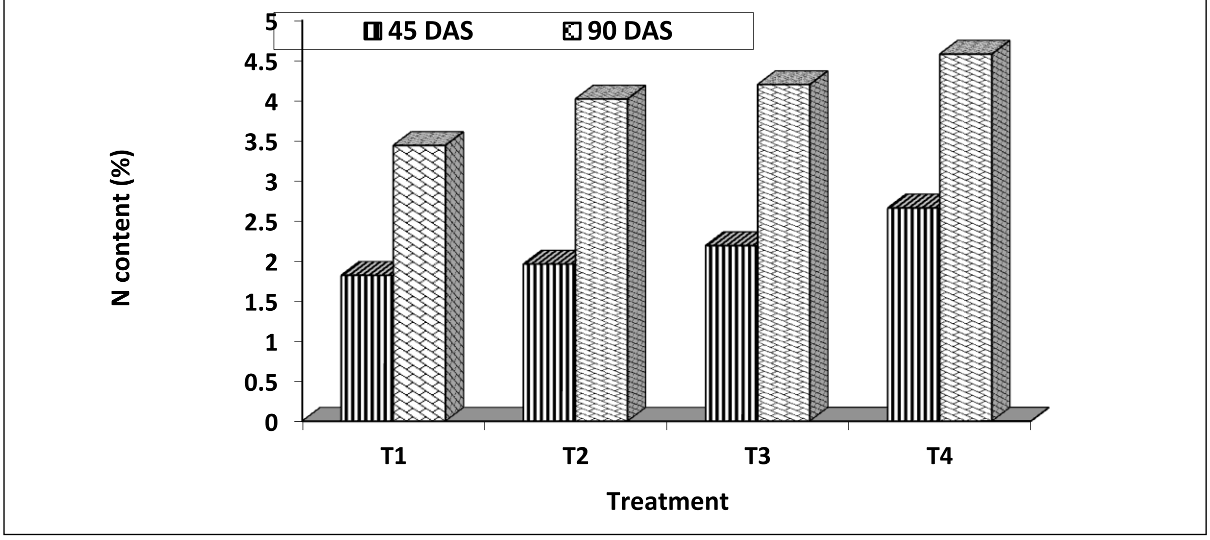
Fig 1.5 Effect of phytoremediation on N content (%) in mustard grain under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 1.6 Effect of phytoremediation on N content (%) in mustard grain under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

ps 90 DAS (4.06%) root had much the higher N accumulation than the 75 DAS (2.16%). The interaction between growing stage and treatment had non-significant role in the N accumulation in mustard grain.

Table 2.1 Effect of phytoremediation on P content (%) in mustard shoot under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.25	0.21	0.23
T2	**RDF+SSP**	0.29	0.24	0.27
T3	**RDF+AM**	0.38	0.27	0.32
T4	**RDF+SSP+AM**	0.46	0.29	0.38
Mean		0.35	0.25	
		SEm±	**CD at 5% level**	
Treatment (T)		0.027	0.080	**SIG**
Stage (S)		0.019	0.057	**SIG**
Interaction (TxS)		0.033	0.098	**NS**

The effects of treatment had significant effect on P accumulation in the mustard plant. T4 (RDF+AM+SSP) was found most prominent effect of P accumulation in the mustard plant (0.38%) where as in T1 (RDF) was observed in the least content (0.23%) in the plant (Table, Fig 2.1 and Appendix 1.1). The stages of the crop growth also have significant effect of P accumulation on the mustard plant. In 45 DAS (0.35%) the plant had much the higher P accumulation than the 90 DAS (0.25%). There was no significant role for interaction which the growing stage and treatment effects on P accumulation in mustard plant.

Table 2.2 Effect of phytoremediation on P content (%) in mustard shoot under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.42	0.19	0.30
T2	**RDF+SSP**	0.44	0.22	0.33
T3	**RDF+AM**	0.47	0.24	0.35
T4	**RDF+SSP+AM**	0.51	0.25	0.38
Mean		0.46	0.23	
		SEm±	**CD at 5% level**	
Treatment (T)		0.058	0.175	**NS**
Stage (S)		0.041	0.124	**SIG**
Interaction (TxS)		0.071	0.214	**NS**

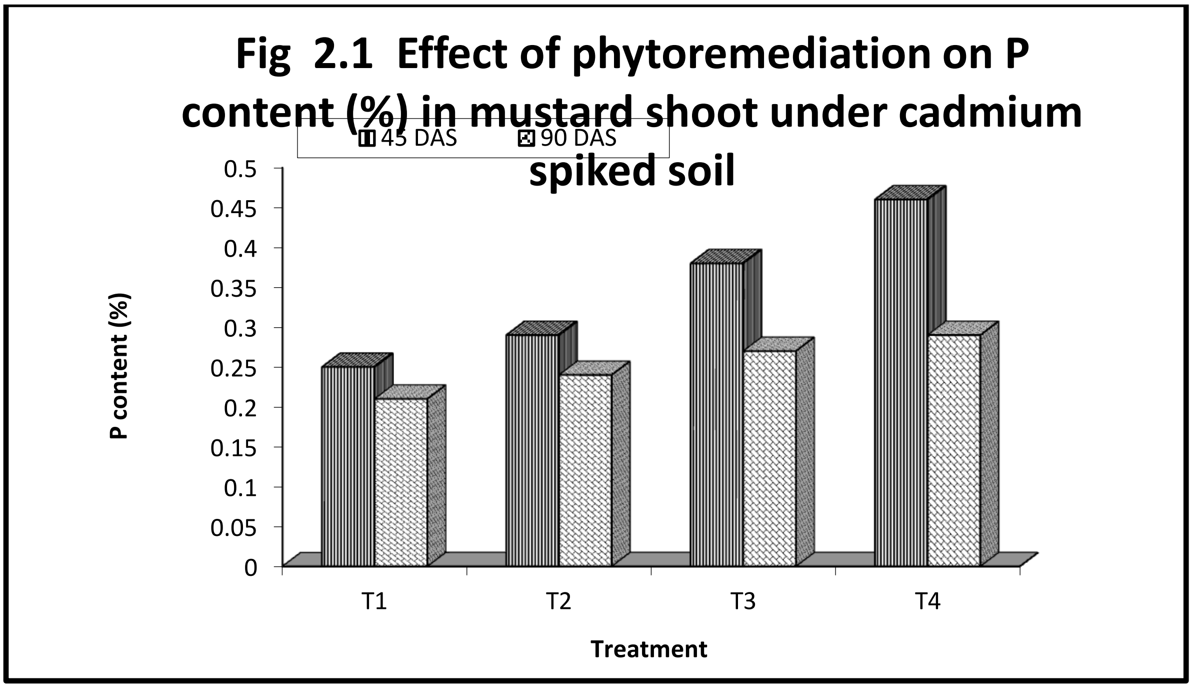


Fig 2.1 Effect of phytoremediation on P content (%) in mustard shoot under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

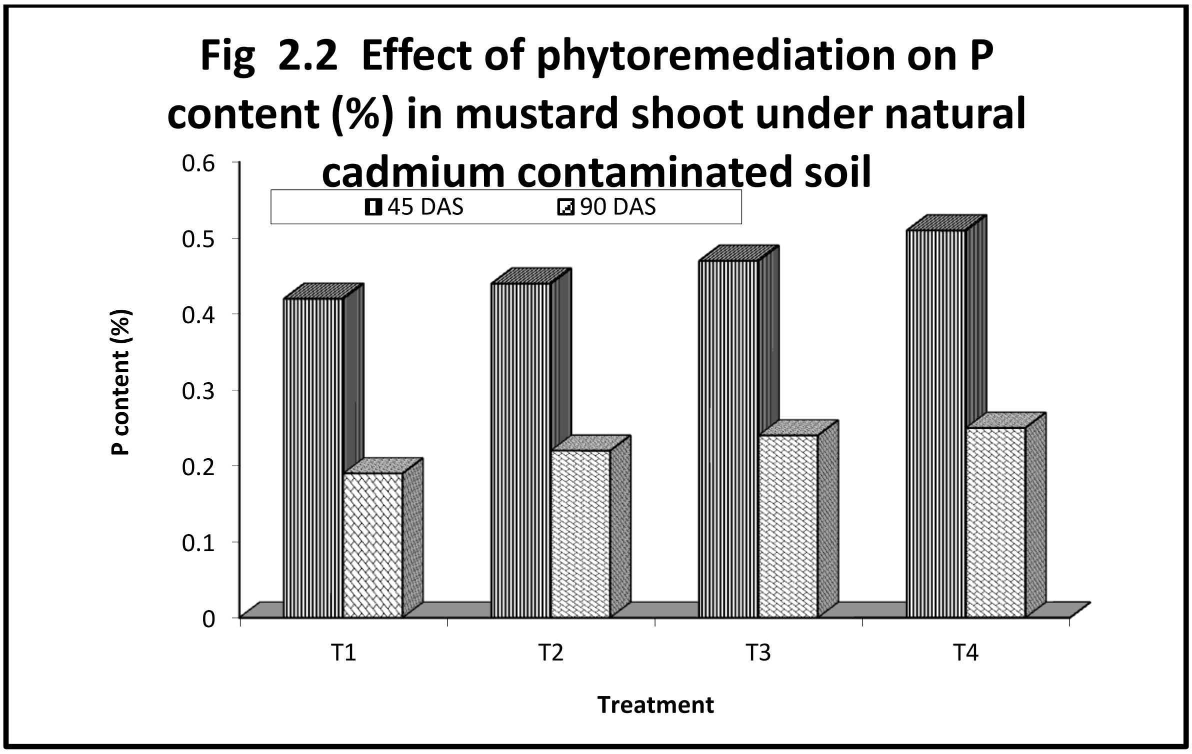


Fig 2.2 Effect of phytoremediation on P content (%) in mustard shoot under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on P accumulation in the mustard plant (Table, Fig 2.2 and Appendix 1.2). The stages of the crop growth had significant effect of P accumulation on the plant. In 45 DAS (0.46%) the plant had much the higher P accumulation than the 90 DAS (0.23%). The interaction between growing stage and treatment had non-significant role in the P accumulation in the mustard plant.

Table 2.3 Effect of phytoremediation on P content (%) in mustard root under cadmium spiked soil

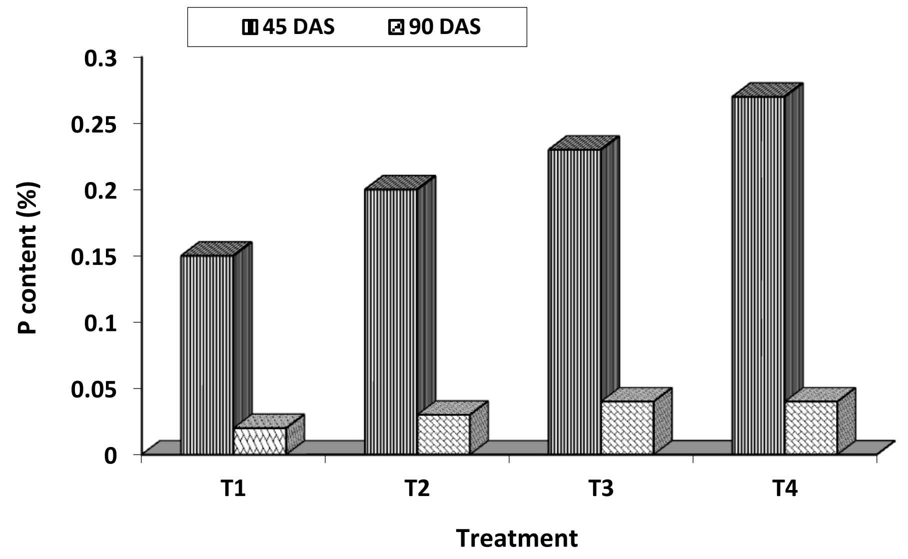
Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.15	0.02	0.09
T2	**RDF+SSP**	0.20	0.03	0.12
T3	**RDF+AM**	0.23	0.04	0.13
T4	**RDF+SSP+AM**	0.27	0.04	0.16
Mean		0.21	0.03	
		SEm±	**CD at 5% level**	
Treatment (T)		0.046	0.138	**NS**
Stage (S)		0.033	0.098	**SIG**
Interaction (TxS)		0.056	0.169	**NS**

In Cd spiked soil, the effects of treatment had non-significant effect on P accumulation in the mustard root (Table, Fig 2.3 and Appendix 1.3). The stages of the crop growth had significant effect of P accumulation on the mustard root. In 90 DAS (0.21%) the plant had much the higher P accumulation than the 45 DAS (0.03%). The interaction effect of growing stage and treatment has non-significant role on the P accumulation in mustard root.

Table 2.4 Effect of phytoremediation on P content (%) in mustard root under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.22	0.04	0.13
T2	**RDF+SSP**	0.25	0.04	0.15
T3	**RDF+AM**	0.26	0.05	0.15
T4	**RDF+SSP+AM**	0.29	0.05	0.17
Mean		0.26	0.05	
		SEm±	**CD at 5% level**	
Treatment (T)		0.053	0.159	**NS**
Stage (S)		0.037	0.112	**SIG**
Interaction (TxS)		0.065	0.195	**NS**

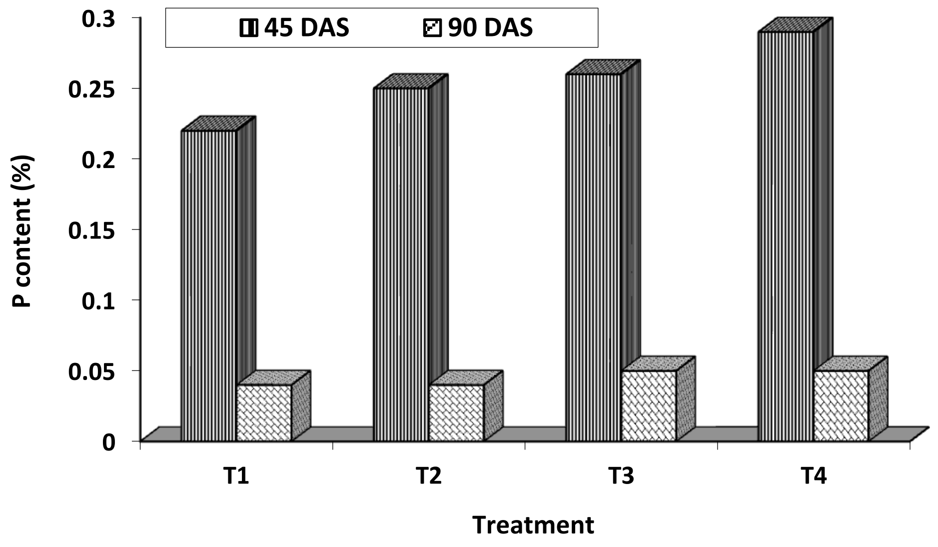
Fig 2.3 Effect of phytoremediation on P content (%) in mustard root under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 2.4 Effect of phytoremediation on P content (%) in mustard root under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Naturally contaminated soil of treatment was found non-significant effect on P accumulation in the mustard (Table, Fig 2.4 and Appendix 1.4). The stages of the crop growth had significant effect of P accumulation on the root. In 45 DAS (0.26%) the root had much the higher P content than the 90 DAS (0.05%). The interaction between growing stage and treatment had non-significant role in the P accumulation in mustard root.

Table 2.5 Effect of phytoremediation on P content (%) in mustard grain under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		75 DAS*	**90 DAS**	**Mean**
T1	**RDF**	1.66	0.85	1.26
T2	**RDF+SSP**	1.86	1.21	1.54
T3	**RDF+AM**	2.23	1.23	1.73
T4	**RDF+SSP+AM**	2.51	1.49	2.00
Mean		2.07	1.20	
		SEm±	**CD at 5% level**	
Treatment (T)		0.221	0.662	**NS**
Stage (S)		0.156	0.468	**SIG**
Interaction (TxS)		0.270	0.810	**NS**

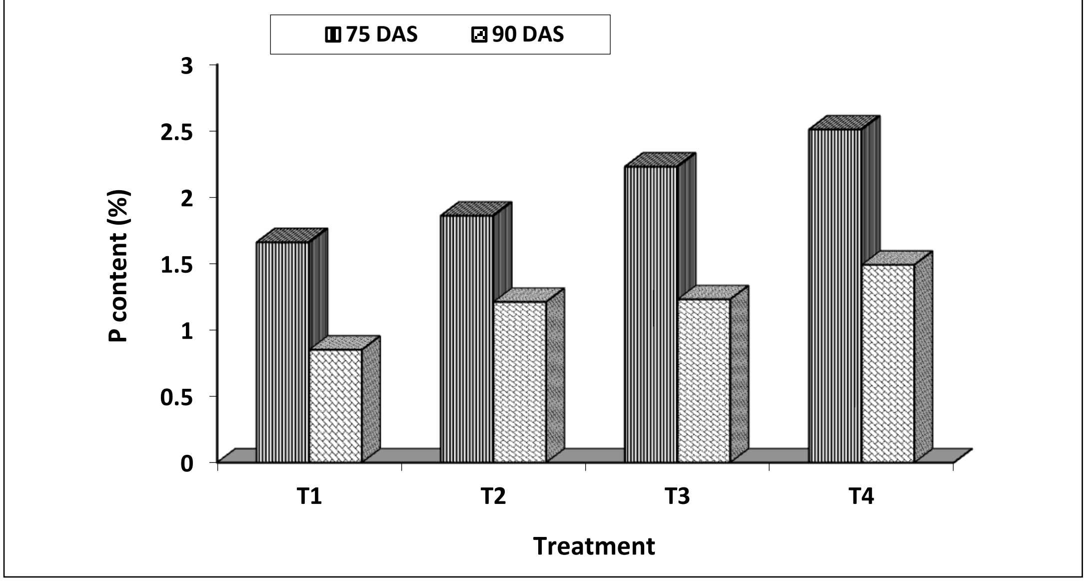
* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S, and Cd) content in the mustard grain.

The effects of treatment had non-significant effect on P accumulation in the mustard grain (Table, Fig 2.5 and Appendix 1.5). The stages of the crop growth had significant effect of P accumulation on the mustard grain. In 75 DAS (2.07%) the plant had much the higher P accumulation than the 90 DAS (1.20%). There was no significant role for interaction of growing stage and treatment in the P content in mustard grain.

Table 2.6 Effect of phytoremediation on P content (%) in mustard grain under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	2.19	0.80	1.50
T2	**RDF+SSP**	2.33	1.03	1.68
T3	**RDF+AM**	2.55	1.10	1.83
T4	**RDF+SSP+AM**	2.73	1.20	1.97
Mean		2.45	1.03	
		SEm±	**CD at 5% level**	
Treatment (T)		0.36	1.07	**NS**
Stage (S)		0.25	0.75	**SIG**
Interaction (TxS)		0.44	1.31	**NS**

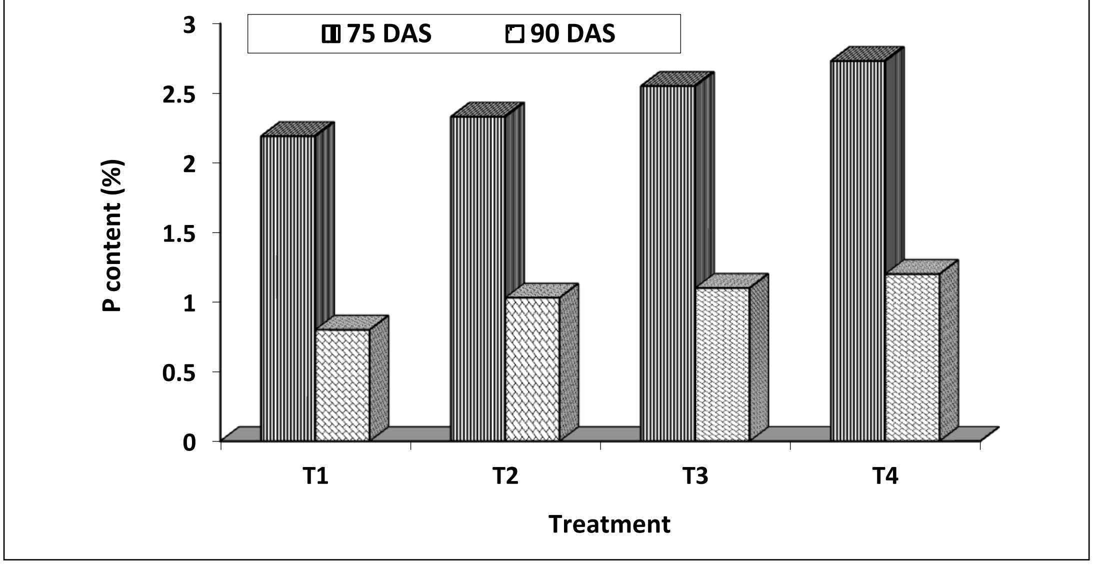
Fig 2.5 Effect of phytoremediation on P content (%) in mustard grain under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 2.6 Effect of phytoremediation on P content (%) in mustard grain under natural cadmium contaminated soil



Treatment

only to

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant role on P accumulation in the mustard grain (Table, Fig 2.6 and Appendix 1.6). The stages of the crop growth had significant effect of P accumulation on the grain. In 90 DAS (2.45%) the root had much the higher P accumulation than the 75 DAS (1.03%). The interaction between growing stage and treatment had non-significant effect in the P accumulation in mustard grain.

Table 3.1 Effect of phytoremediation on K content (%) in mustard shoot under cadmium spiked soil

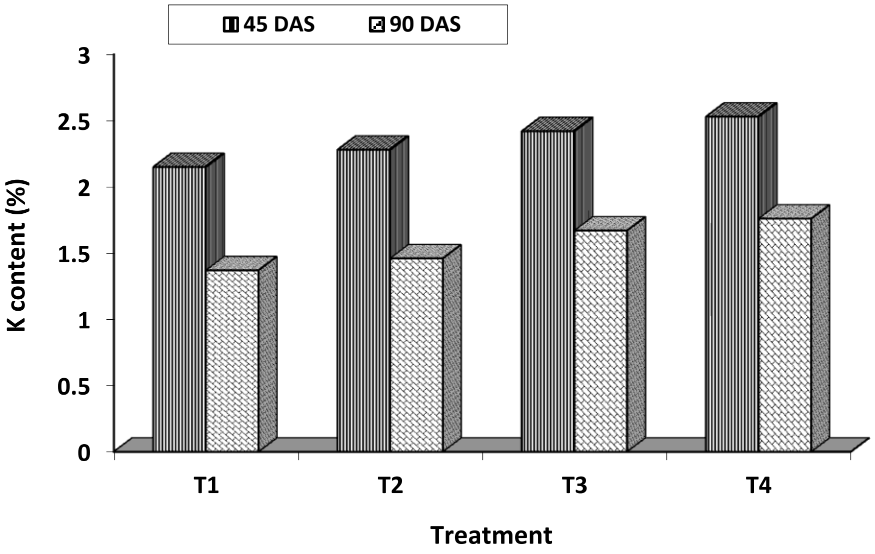
Treatment		**Stage of plant growth (mustard)**		
		45 Days	**90 Days**	**Mean**
T1	**RDF**	2.15	1.37	1.76
T2	**RDF+SSP**	2.28	1.46	1.87
T3	**RDF+AM**	2.42	1.67	2.04
T4	**RDF+SSP+AM**	2.53	1.76	2.15
Mean		2.35	1.57	
		SEm±	**CD at 5% level**	
Treatment (T)		0.195	0.586	**NS**
Stage (S)		0.138	0.414	**SIG**
Interaction (TxS)		0.239	0.717	**NS**

The effects of treatment had non-significant effect on K accumulation in the mustard plant (Table, Fig 3.1 and Appendix 1.1). The stages of the crop growth had significant effect of K accumulation on the mustard plant. In 45 DAS (2.35%) the plant had much the higher K accumulation than the 90 DAS (1.57%). There was no significant effect on the interaction of growing stage and treatment in the K accumulation of in mustard plant.

Table 3.2 Effect of phytoremediation on K content (%) in mustard shoot under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	6.62	4.44	5.53
T2	**RDF+SSP**	8.19	5.15	6.67
T3	**RDF+AM**	8.38	5.70	7.04
T4	**RDF+SSP+AM**	9.10	6.13	7.61
Mean		8.07	5.36	
		SEm±	**CD at 5% level**	
Treatment (T)		0.228	0.683	**NS**
Stage (S)		0.161	0.483	**SIG**
Interaction (TxS)		0.279	0.837	**NS**

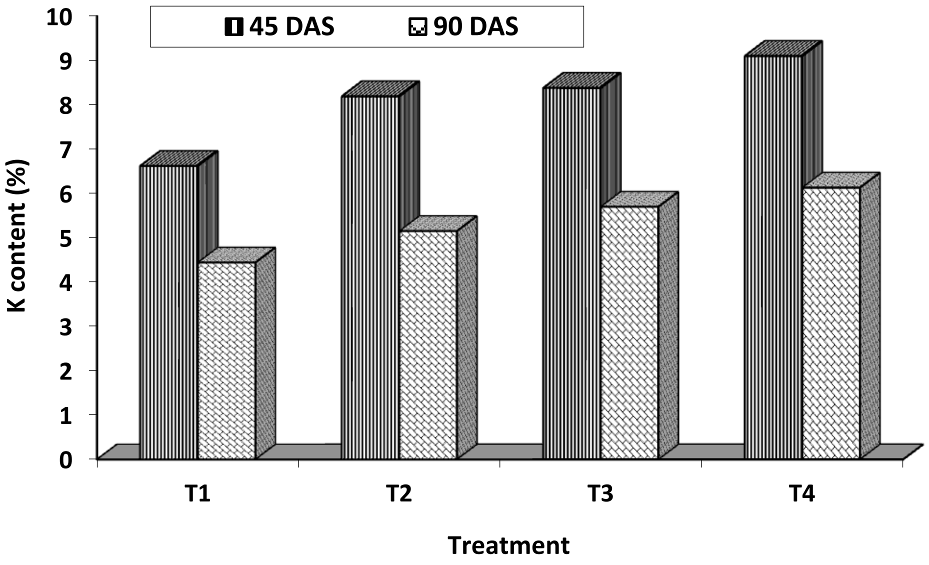
Fig 3.1 Effect of phytoremediation on K content (%) in mustard shoot under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 3.2 Effect of phytoremediation on K content (%) in mustard shoot under natural cadmium contaminated soil



The effects of treatment had non-significant effect on K accumulation in the mustard shoot (Table, Fig 3.2 and Appendix 1.2). The stages of the crop growth had significant effect of K accumulation on the plant. In 45 DAS (8.07%) the plant had much the higher K accumulation than the 90 DAS (5.36%). The interaction between growing stage and treatment had non-significant role in the K accumulation in mustard plant.

Table 3.3 Effect of phytoremediation on K content (%) in mustard root under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.31	0.46	0.88
T2	**RDF+SSP**	1.46	0.56	1.01
T3	**RDF+AM**	1.53	0.70	1.12
T4	**RDF+SSP+AM**	1.71	0.73	1.22
Mean		1.50	0.61	
		SEm±	**CD at 5% level**	
Treatment (T)		0.223	0.669	**NS**
Stage (S)		0.158	0.473	**SIG**
Interaction (TxS)		0.273	0.819	**NS**

The effects of treatment had non-significant effect on K content in the mustard root (Table, Fig 3.3 and Appendix 1.3). The stages of the crop growth had significant effect of K content on the mustard root. In 45 DAS (1.50%) the plant had much the higher K content than the 90 DAS (0.61%). There was no significant role which the interaction of growing stage and treatment in the K content of in mustard root.

Table 3.4 Effect of phytoremediation on K content (%) in mustard plant root under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.43	0.66	1.05
T2	**RDF+SSP**	1.72	0.76	1.24
T3	**RDF+AM**	1.85	0.81	1.33
T4	**RDF+SSP+AM**	2.00	0.87	1.43

Mean	1.75	0.78	
	SEm±	**CD at 5% level**	
Treatment (T)	0.25	0.74	**NS**
Stage (S)	0.17	0.52	**SIG**
Interaction (TxS)	0.30	0.90	**NS**

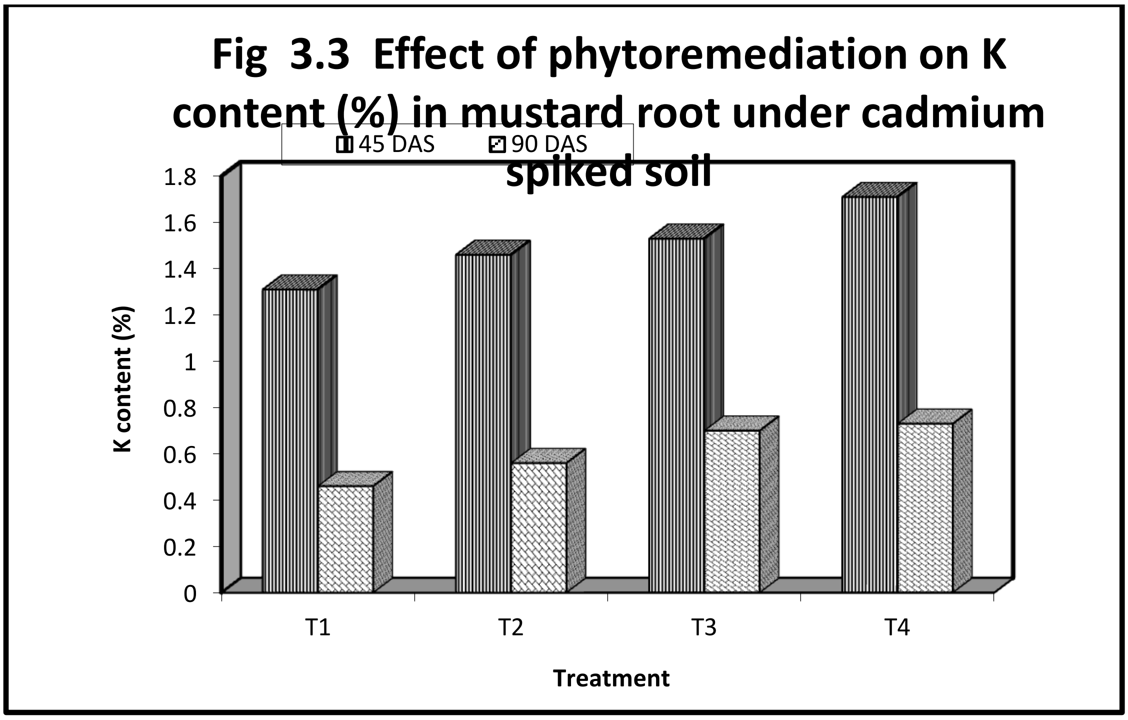


Fig 3.3 Effect of phytoremediation on K content (%) in mustard root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

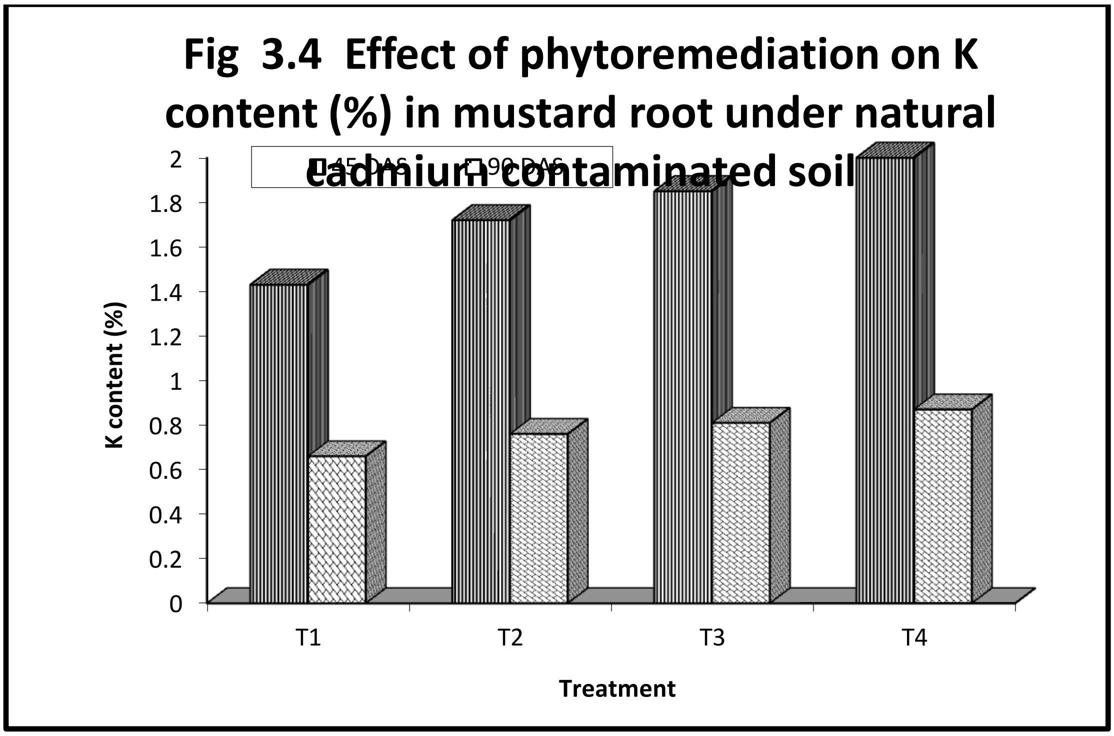


Fig 3.4 Effect of phytoremediation on K content (%) in mustard root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on K content in the mustard (Table, Fig 3.4 and Appendix 1.4). The stages of the crop growth had significant effect of K content on the root. In 45 DAS (1.75%) the root had much the higher K content than the 90 DAS (0.78%). The interaction between growing stage and treatment had non-significant role in the K content in mustard root.

Table 3.5 Effect of phytoremediation on K content (%) in mustard grain under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.74	0.66	1.20
T2	**RDF+SSP**	1.90	0.83	1.36
T3	**RDF+AM**	2.00	0.87	1.44
T4	**RDF+SSP+AM**	2.26	0.93	1.59
Mean		1.98	0.82	
		SEm±	**CD at 5% level**	
Treatment (T)		0.290	0.868	**NS**
Stage (S)		0.205	0.614	**SIG**
Interaction (TxS)		0.355	1.063	**NS**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S, and Cd) content in the mustard grain.

The effects of treatment had non-significant effect on K content in the mustard grain (Table, Fig 3.5 and Appendix 1.5). The stages of the crop growth had significant effect of K content on the mustard grain. In 75 DAS (1.98%) the plant had much the higher K content than the 90 DAS (0.82%). There was no significant role which the interaction of growing stage and treatment in the K accumulation of in mustard grain.

Table 3.6 Effect of phytoremediation on K content (%) in mustard grain under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	2.29	0.80	1.55
T2	**RDF+SSP**	2.34	0.86	1.60
T3	**RDF+AM**	2.43	0.92	1.68
T4	**RDF+SSP+AM**	2.53	0.97	1.75
Mean		2.40	0.89	
		SEm±	**CD at 5% level**	

Treatment (T)	0.377	1.131	**NS**
Stage (S)	0.267	0.800	**SIG**
Interaction (TxS)	0.462	1.386	**NS**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S, and Cd) content in the mustard grain.

The effects of treatment had non-significant effect on K accumulation in the mustard grain (Table, Fig 3.6 and Appendix 1.6). The stages of the crop growth had significant effect of K accumulation on the grain. In 75 DAS (2.40%) the root had much the higher K accumulation than the 90 DAS (0.89%). The interaction between growing stage and treatment had non-significant role in the K accumulation in mustard grain.

Table 4.1 Effect of phytoremediation on S content (%) in mustard shoot under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.38	0.18	0.28
T2	**RDF+SSP**	0.42	0.30	0.36
T3	**RDF+AM**	0.45	0.31	0.38
T4	**RDF+SSP+AM**	0.81	0.41	0.61
Mean		0.52	0.30	
		SEm±	**CD at 5% level**	
Treatment (T)		0.061	0.183	**SIG**
Stage (S)		0.043	0.129	**SIG**
Interaction (TxS)		0.075	0.224	**NS**

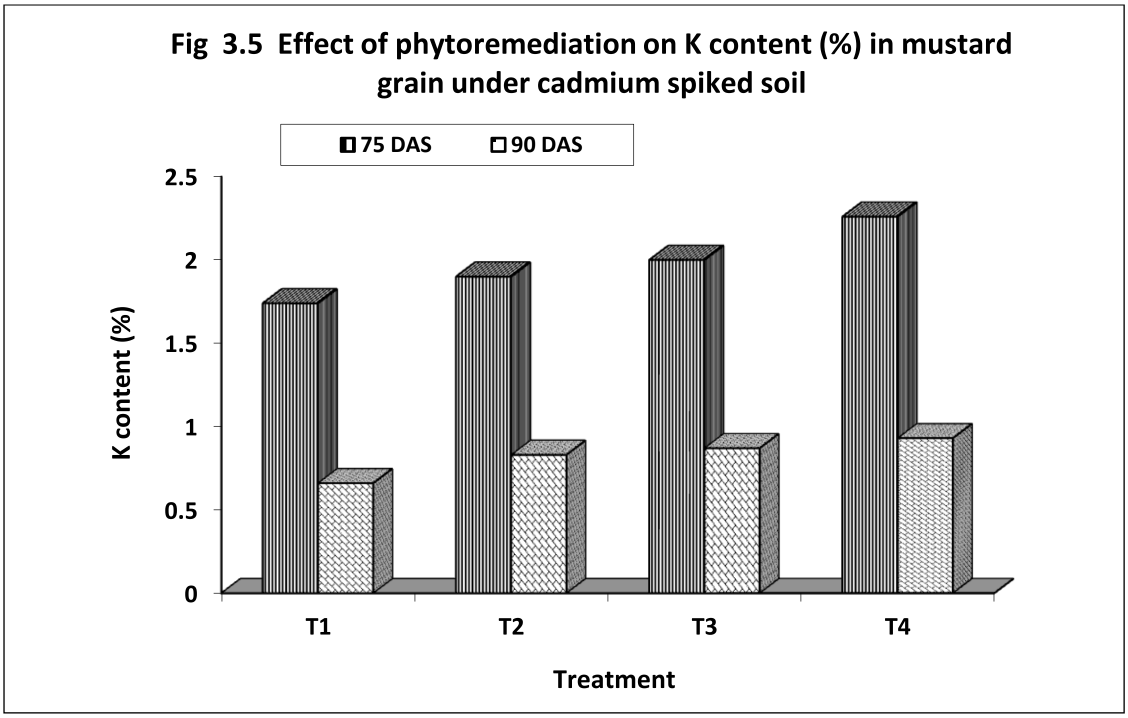


Fig 3.5 Effect of phytoremediation on K content (%) in mustard grain under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

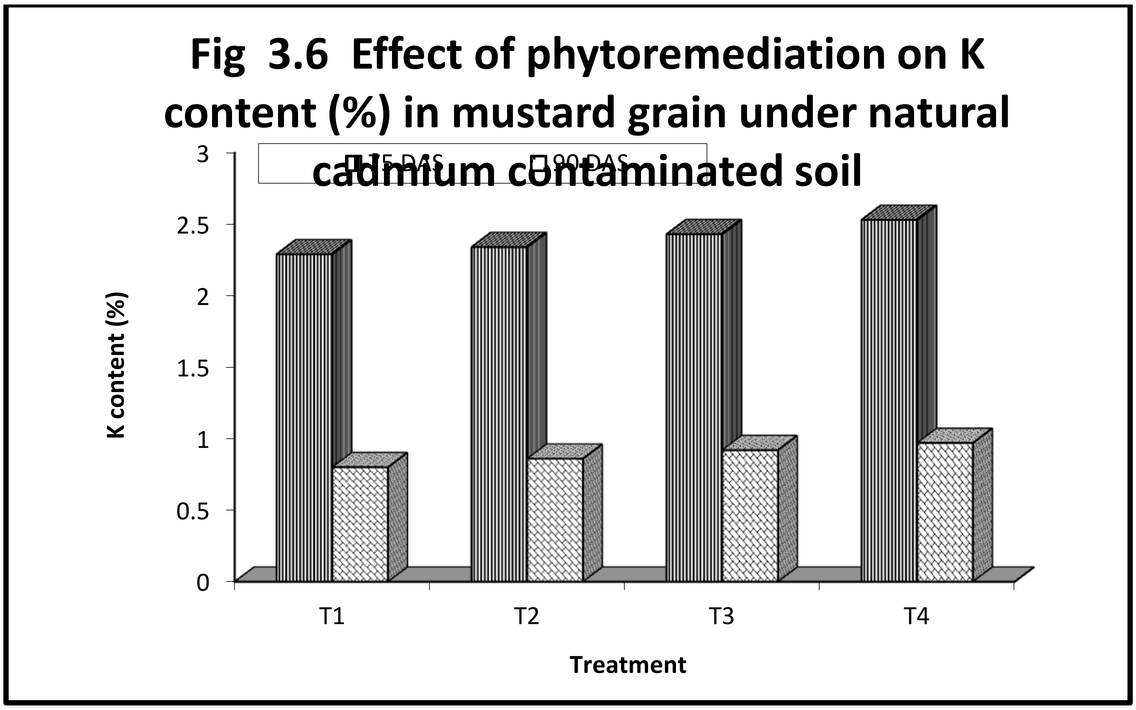


Fig 3.6 Effect of phytoremediation on K content (%) in mustard grain under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on S content in the mustard plant which was observed 0.38% (T3) and 0.36% (T2) respectively (Table, Fig 4.1 and Appendix 1.1). The T4 (RDF+AM+SSP) was found most prominent effect S content in the mustard plant (0.61%) whereas T1 (RDF) was observed in the least content (0.28%) in the plant. The stages of the crop growth had significant effect of S content on the mustard plant. In 45 DAS (0.52%) the plant have much the higher S content than the 90 DAS (0.30%). There was no significant role which the interaction of growing stage and treatment in the S content of in mustard plant.

Table 4.2 Effect of phytoremediation on S content (%) in mustard shoot under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.39	0.19	0.29
T2	**RDF+SSP**	0.47	0.33	0.40
T3	**RDF+AM**	0.72	0.34	0.53
T4	**RDF+SSP+AM**	0.83	0.39	0.61
Mean		0.60	0.31	
		SEm±	**CD at 5% level**	
Treatment (T)		0.078	0.234	**SIG**
Stage (S)		0.055	0.165	**SIG**
Interaction (TxS)		0.095	0.286	**NS**

The effects of treatment had significant effect on S content in the mustard plant which was observed 0.53% (T3) and 0.40% (T2) respectively (Table, Fig 4.2 and Appendix 1.2). The T4 (RDF+AM+SSP) was found most prominent effect S content in the plant (0.61%) whereas T1 (RDF) was found in the least content (0.29%) in the plant. The stages of the crop growth had significant effect of S content on the plant. In 45 DAS (0.60%) the plant had much the higher S content than the 90 DAS (0.31%). The interaction between growing stage and treatment was non-significant role in the S accumulation in mustard plant.

Table 4.3 Effect of phytoremediation on S content (%) in mustard root under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.10	0.24	0.17
T2	**RDF+SSP**	0.11	0.31	0.21
T3	**RDF+AM**	0.13	0.38	0.25

T4	**RDF+SSP+AM**	0.13	0.38	0.26
Mean		0.12	0.33	
		SEm±	**CD at 5% level**	
Treatment (T)		0.053	0.159	**NS**
Stage (S)		0.038	0.113	**SIG**
Interaction (TxS)		0.065	0.195	**NS**

The effects of treatment had non-significant effect on S content in the mustard root (Table, Fig 4.3 and Appendix 1.3). The stages of the crop growth had significant effect of S content on the mustard root. In 90 DAS (0.33%) the plant had much the higher S content than the 45 DAS (0.33%). The interaction between growing stage and treatment had non-significant role in the S accumulation in mustard plant.

Table 4.4 Effect of phytoremediation on S content (%) in mustard root under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.11	0.27	0.19
T2	**RDF+SSP**	0.16	0.27	0.22
T3	**RDF+AM**	0.17	0.30	0.24
T4	**RDF+SSP+AM**	0.50	0.36	0.43
Mean		0.24	0.30	
		SEm±	**CD at 5% level**	
Treatment (T)		0.034	0.102	**SIG**
Stage (S)		0.024	0.072	**NS**
Interaction (TxS)		0.042	0.125	**SIG**

The effects of treatment had significant effect on S content in the mustard root which was found 0.24% (T3) and 0.22% (T2) respectively (Table, Fig 4.4 and Appendix 1.4). The T4 (RDF+AM+SSP) was found most prominent effect S content in the root (0.43%) whereas T1 (RDF) was found in the least content (0.19%) in the mustard root. The stages of the crop growth had non-significant effect of S content on the root. In this study the interaction between growing stage and treatment had significant role on

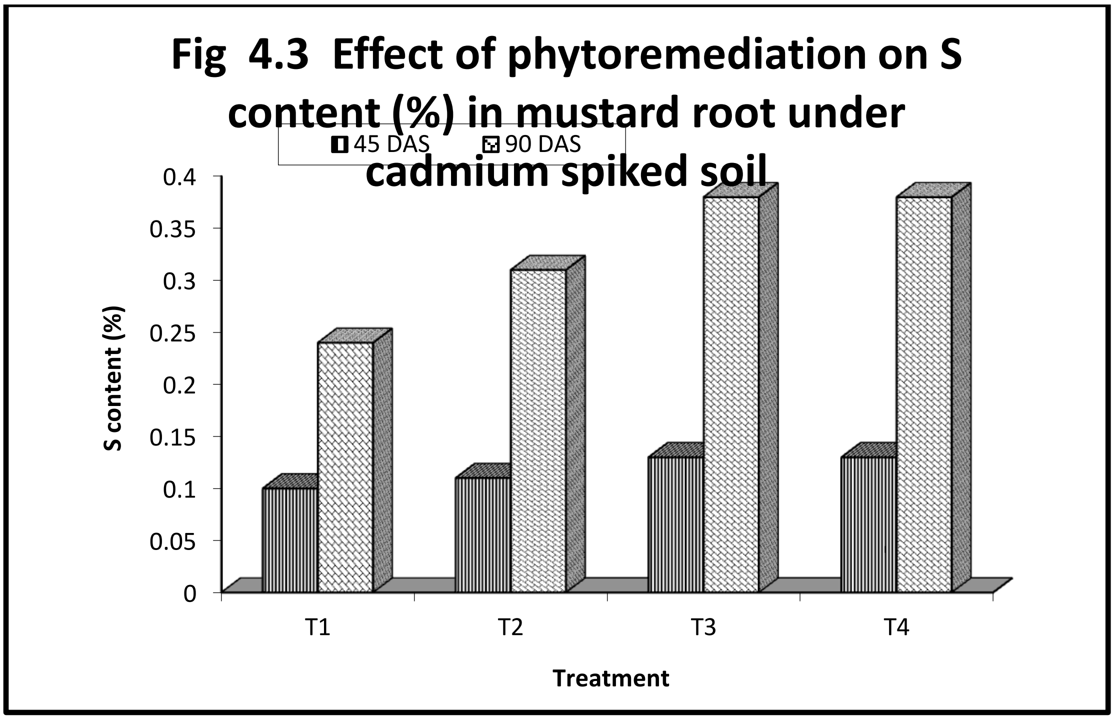


Fig 4.3 Effect of phytoremediation on S content (%) in mustard root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

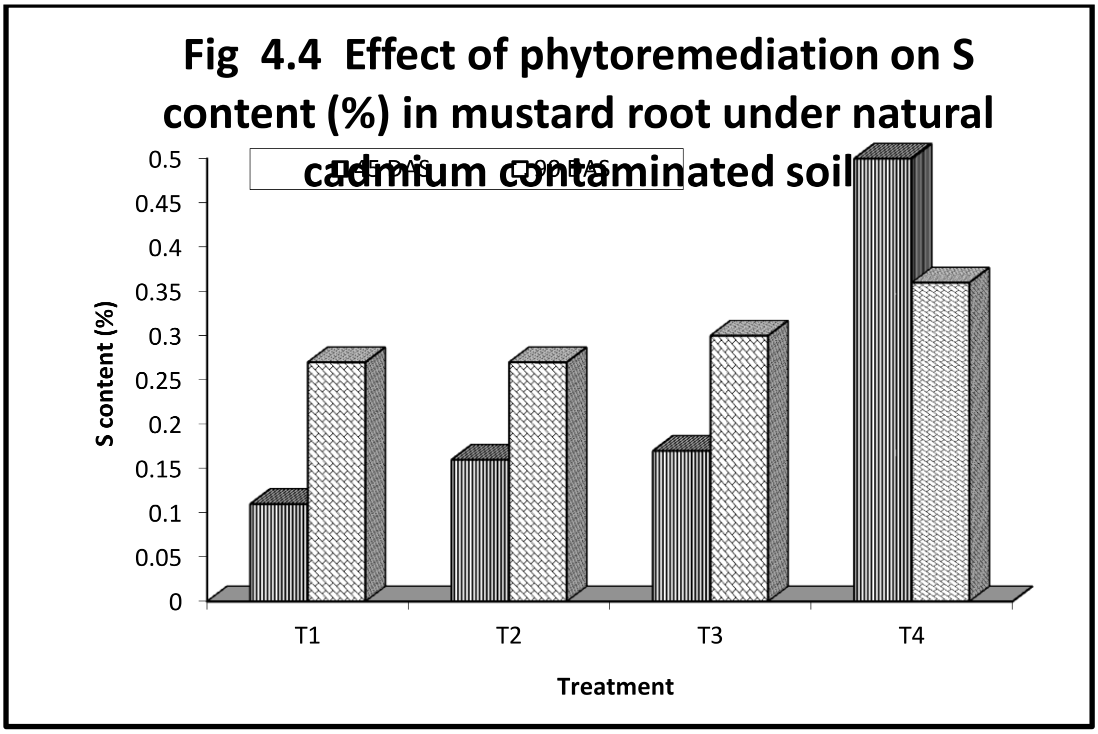


Fig 4.4 Effect of phytoremediation on S content (%) in mustard root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

mustard root S accumulation in naturally cadmium contaminated. In the interaction the T4 (0.50%) treatment in 45 DAS was found superior than other treatment combination with the growing stage of mustard plant on S accumulation.

Table 4.5 Effect of phytoremediation on S content (%) in mustard grain under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.00	0.81	0.90
T2	**RDF+SSP**	1.11	1.14	1.13
T3	**RDF+AM**	1.19	1.15	1.17
T4	**RDF+SSP+AM**	1.38	1.14	1.26
Mean		1.17	1.06	
		SEm±	**CD at 5% level**	
Treatment (T)		0.039	0.117	**SIG**
Stage (S)		0.028	0.083	**SIG**
Interaction (TxS)		0.048	0.143	**NS**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S, and Cd) content in the mustard grain.

The effects of treatment had significant effect on S content in the mustard grain which was observed 1.17% (T3) and 1.13% (T2) respectively (Table, Fig 4.5 and Appendix 1.5). The T4 (RDF+AM+SSP) was found most prominent effect S content in the mustard grain (1.26%) where as T1 (RDF) was found in the least content (0.90%) in the grain. The stages of the crop growth had significant effect of S content on the mustard grain. In 75 DAS (1.17%) the plant had much the higher S content than the 90 DAS (1.06%). There was no significant role for interaction which the growing stage and treatment in the K content of in mustard grain.

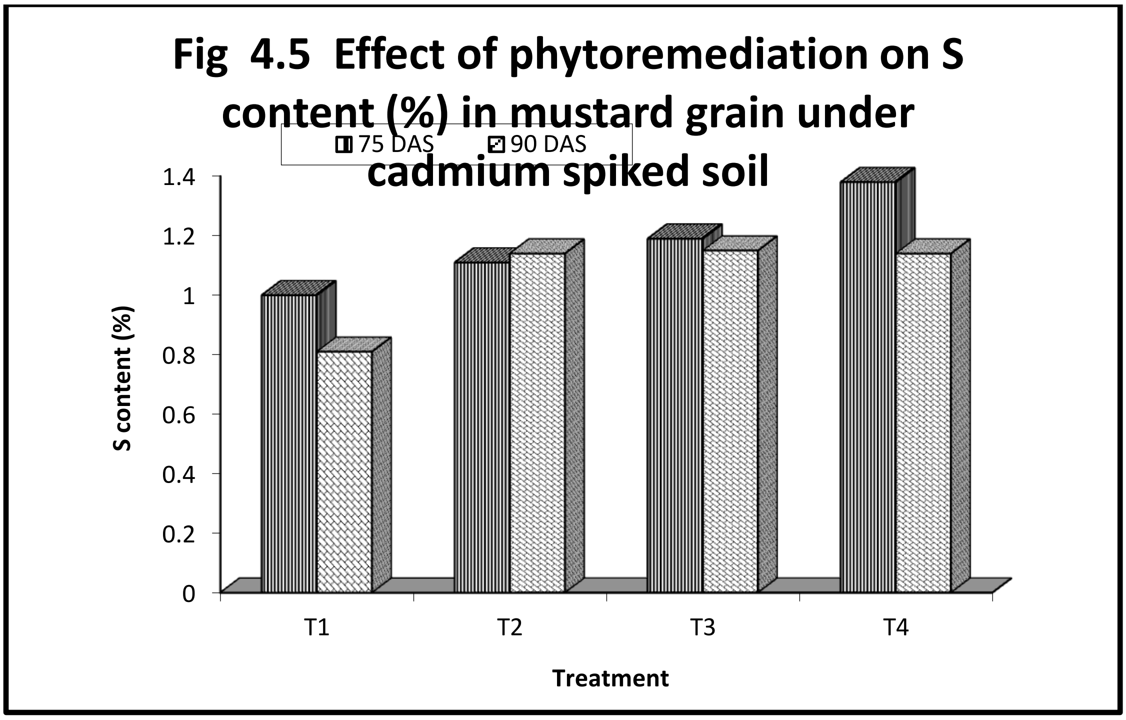


Fig 4.5 Effect of phytoremediation on S content (%) in mustard grain under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

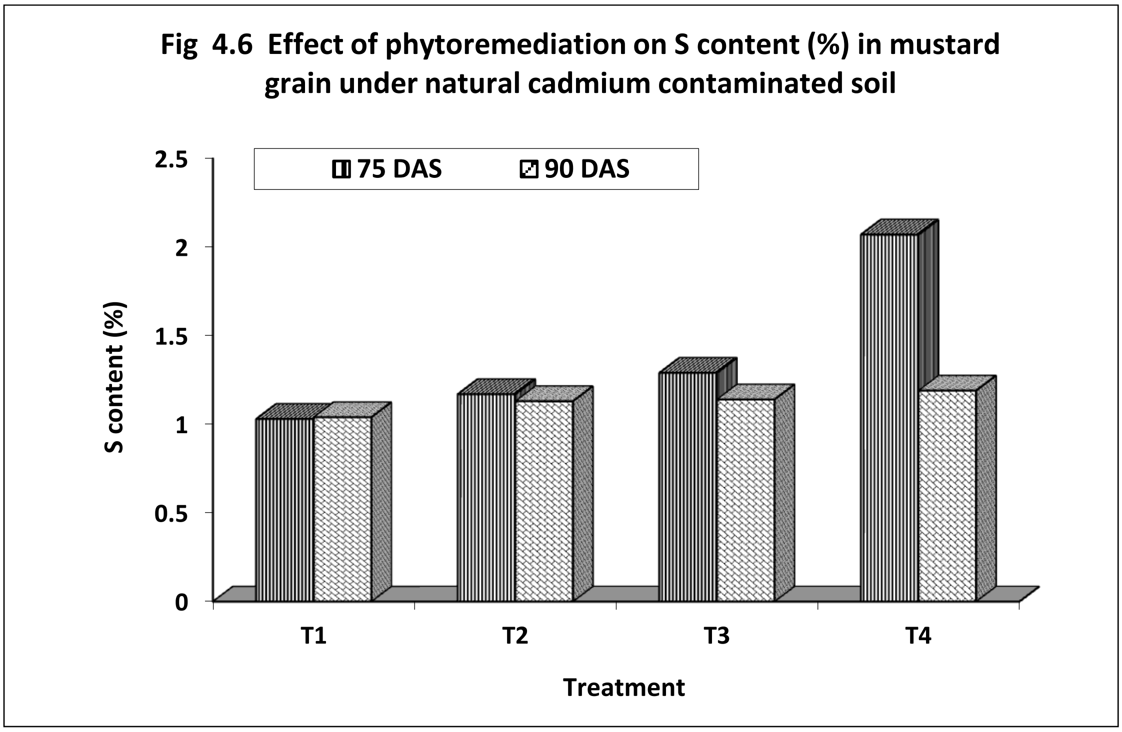


Fig 4.6 Effect of phytoremediation on S content (%) in mustard grain under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 4.6 Effect of phytoremediation on S content (%) in mustard grain under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	1.03	1.04	1.03
T2	**RDF+SSP**	1.17	1.13	1.15
T3	**RDF+AM**	1.29	1.14	1.22
T4	**RDF+SSP+AM**	2.07	1.19	1.63
Mean		1.39	1.13	
		SEm±	**CD at 5% level**	
Treatment (T)		0.112	0.337	**SIG**
Stage (S)		0.079	0.238	**SIG**
Interaction (TxS)		0.138	0.413	**SIG**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S, and Cd) content in the mustard grain.

The effects of treatment had significant effect on S content in the mustard grain which was found 1.22% (T3) and 1.15% (T2), respectively (Table, Fig 4.6 and Appendix 1.6) whereas T4 (RDF+AM+SSP) was found most prominent effect on S content in the grain (1.63%) where as T1 (RDF) was found in the least content (1.03%) in the mustard grain. The stages of the crop growth had significant effect of S content on the grain. In 75 DAS (1.39%) the grain had much the higher S content than the 90 DAS (1.13%). In this study the interaction between growing stage and treatment due to S accumulation AM addition have significant role on mustard grain S accumulation in naturally cadmium contaminated soil. In the interaction the T4 (2.07%) treatment in 45 DAS was found superior than other treatment combination with the growing stage of mustard plant on S accumulation.

Table 5.1 Effect of phytoremediation on Cd content (ppm) in mustard shoot under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	90.9	139.2	115.1
T2	**RDF+SSP**	103.3	158.5	130.9
T3	**RDF+AM**	111.9	188.9	150.4

T4	**RDF+SSP+AM**	110.2	131.1	120.7
Mean		104.1	154.4	
		SEm±	**CD at 5% level**	
Treatment (T)		13.5	40.6	**NS**
Stage (S)		9.6	28.7	**SIG**
Interaction (TxS)		16.6	49.7	**NS**

In Cd spiked soil, the effects of treatment had non-significant effect on Cd content in the mustard plant (Table, Fig 5.1 and Appendix 1.1). The stages of the crop growth showed significant effect of Cd content in the mustard crop. In 90 DAS (154.4 ppm) the plant have much the higher Cd content than the 45 DAS (104.1 ppm). With the plant growth succession in terms of phytoremediation has significant contribution to increase the Cd accumulation by the mustard shoot. There was no significant role which the interaction of growing stage and treatment on the Cd content in mustard crop.

Table 5.2 Effect of phytoremediation on Cd content (ppm) in mustard shoot under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 Days	**90 Days**	**Mean**
T1	**RDF**	1.60	3.20	2.40
T2	**RDF+SSP**	1.87	3.27	2.57
T3	**RDF+AM**	1.89	3.73	2.81
T4	**RDF+SSP+AM**	3.93	4.07	4.00
Mean		2.32	3.57	
		SEm±	**CD at 5% level**	
Treatment (T)		0.35	1.05	**SIG**
Stage (S)		0.25	0.75	**SIG**
Interaction (TxS)		0.43	1.29	**NS**

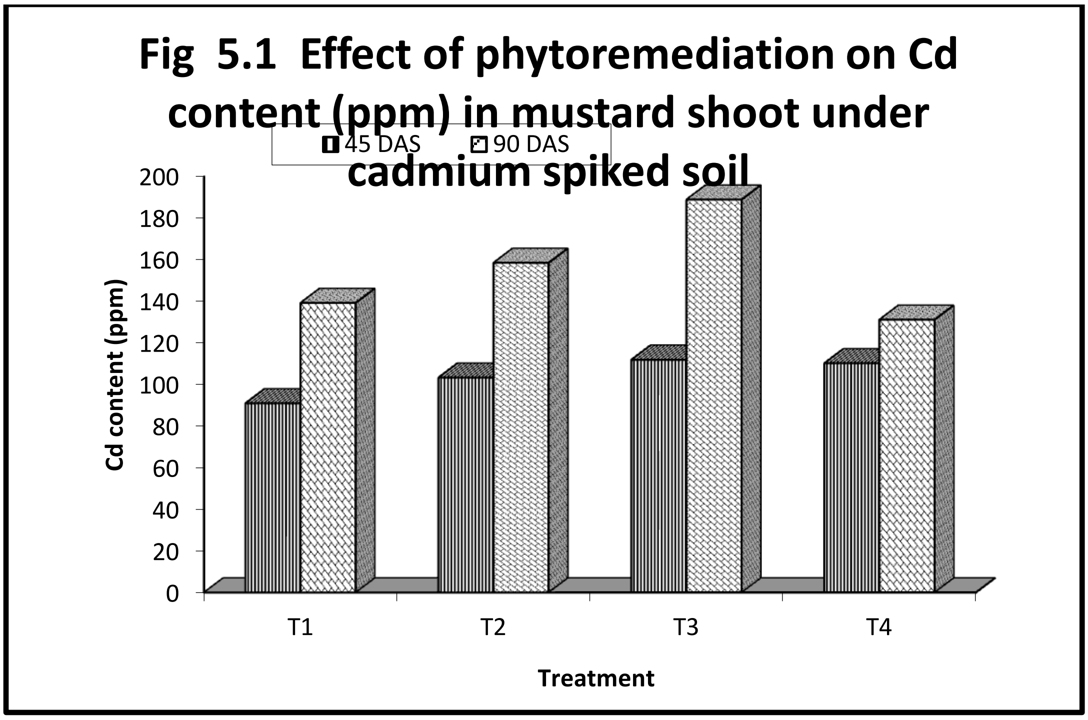


Fig 5.1 Effect of phytoremediation on Cd content (ppm) in mustard shoot under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

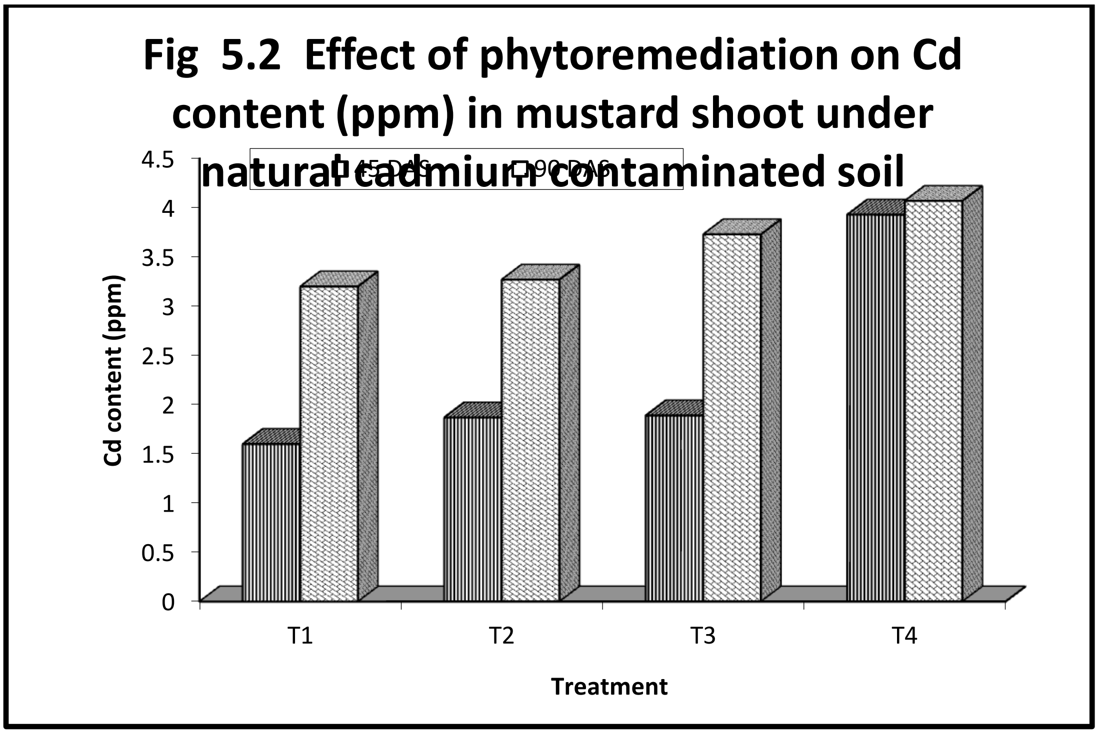


Fig 5.2 Effect of phytoremediation on Cd content (ppm) in mustard shoot under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

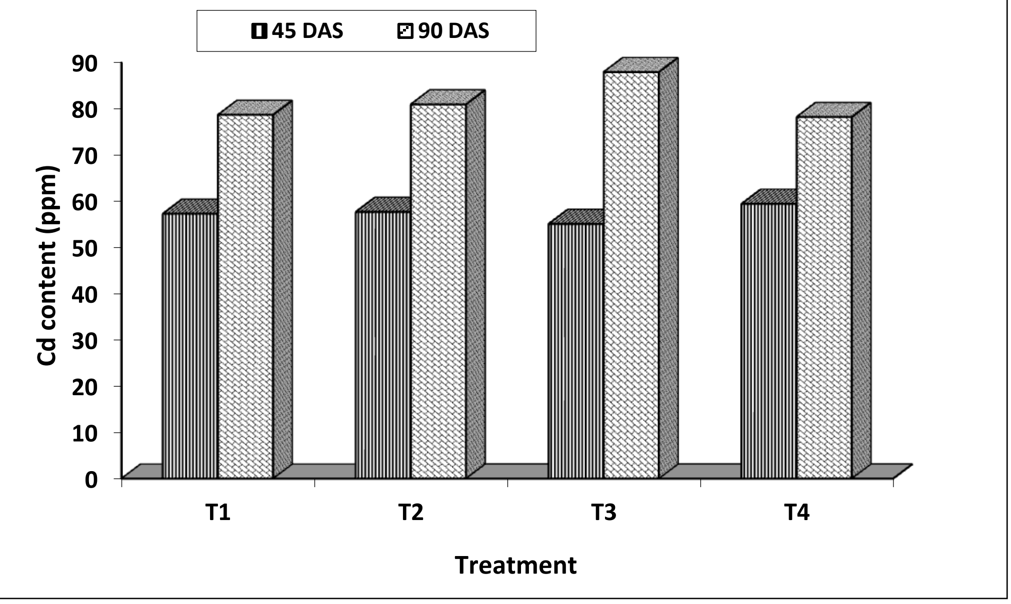
Under naturally Cd contaminated soil, the effects of treatment showed significant effect on Cd content in the mustard shoot which was observed 2.81 ppm (T3) and 2.57 ppm (T2) respectively (Table, Fig 5.2 and Appendix 1.2). The T4 (RDF+AM+SSP) was found most prominent effect of Cd content in the plant (4.0 ppm) whereas T1 (RDF) was found in the least content (2.40 ppm) in the plant. The stages of the crop growth had significant effect of Cd content in the plant. In 90 DAS (3.57 ppm) the plant had much the higher Cd content than the 45 DAS (2.32 ppm). The interaction between growing stage and treatment showed non-significant role in the Cd content in mustard plant.

Table 5.3 Effect of phytoremediation on Cd content (ppm) in mustard root under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	57.33	78.73	68.03
T2	**RDF+SSP**	57.73	81.00	69.37
T3	**RDF+AM**	55.13	88.00	71.57
T4	**RDF+SSP+AM**	59.47	78.27	68.87
Mean		57.42	81.50	
		SEm±	**CD at 5% level**	
Treatment (T)		6.17	18.48	**NS**
Stage (S)		4.36	13.07	**SIG**
Interaction (TxS)		7.55	22.64	**NS**

The effects of treatment showed non-significant effect on Cd content in the mustard root (Table, Fig 5.3 and Appendix 1.3). The stages of the crop growth had significant effect of Cd content on the mustard root. In 90 DAS (81.50 ppm) the plant had much higher Cd content than the 45 DAS (57.42 ppm). The interaction between growing stage and treatment had non-significant role in the Cd accumulation in mustard plant.

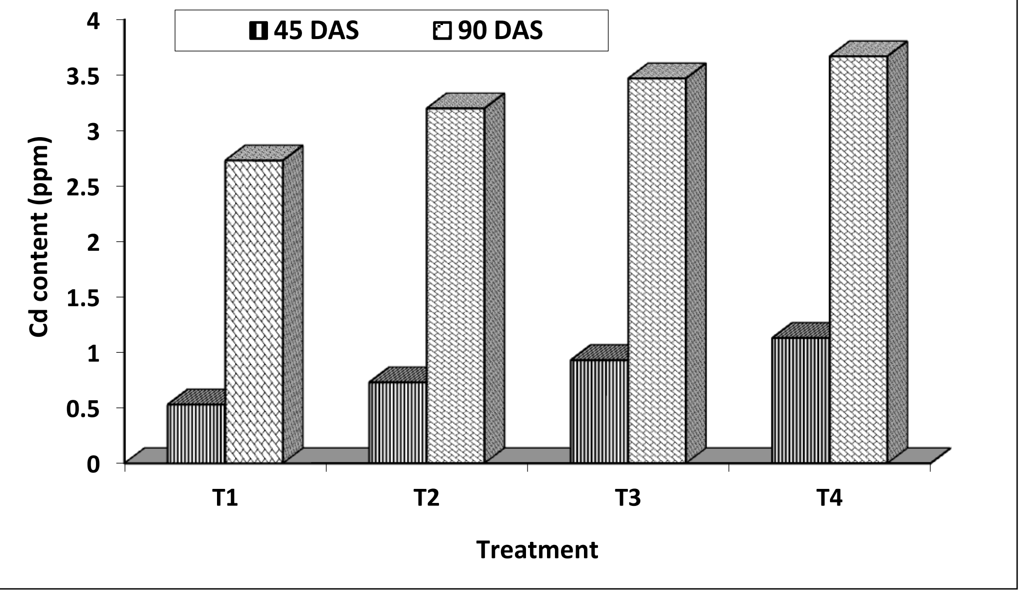
Fig 5.3 Effect of phytoremediation on Cd content (ppm) in mustard root under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 5.4 Effect of phytoremediation on Cd content (ppm) in mustard root under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 5.4 Effect of phytoremediation on Cd content (ppm) in mustard root under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.53	2.73	1.63
T2	**RDF+SSP**	0.73	3.20	1.97
T3	**RDF+AM**	0.93	3.47	2.20
T4	**RDF+SSP+AM**	1.13	3.67	2.40
Mean		0.83	3.27	
		SEm±	**CD at 5% level**	
Treatment (T)		0.61	1.83	**NS**
Stage (S)		0.43	1.29	**SIG**
Interaction (TxS)		0.75	2.24	**NS**

The effects of treatment showed non-significant effect on Cd content in the mustard root (Table, Fig 5.4 and Appendix 1.4). The stages of the crop growth had significant effect of Cd content on the root. In 90 DAS (3.27 ppm) the root had much the higher Cd content than the 45 DAS (0.83 ppm). The interaction between growing stage and treatment showed non-significant role in the Cd content in mustard plant.

Table 5.5 Effect of phytoremediation on Cd content (ppm) in mustard grain under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	14.47	27.53	21.00
T2	**RDF+SSP**	15.20	26.60	20.90
T3	**RDF+AM**	16.13	20.40	18.27
T4	**RDF+SSP+AM**	17.87	35.53	26.70
Mean		15.92	27.52	
		SEm±	**CD at 5% level**	
Treatment (T)		3.14	9.41	**NS**
Stage (S)		2.22	6.66	**SIG**
Interaction (TxS)		3.85	11.53	**NS**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S, and Cd) content in the mustard grain.

The effects of treatment had non-significant effect on Cd content in the mustard grain (Table, Fig 5.5 and Appendix 1.5). The stages of the crop growth had significant effect of Cd content on the mustard grain. In 90 DAS (27.52 ppm) the plant had much higher Cd content than the 75 DAS (15.92 ppm). There was no significant role which the interaction growing stage and treatment in the Cd content of in mustard grain.

Table 5.6 Effect of phytoremediation on Cd content (ppm) in mustard grain under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		75 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.20	0.33	0.27
T2	**RDF+SSP**	0.27	0.67	0.47
T3	**RDF+AM**	0.40	0.73	0.57
T4	**RDF+SSP+AM**	0.53	0.67	0.60
Mean		0.35	0.60	
		SEm±	**CD at 5% level**	
Treatment (T)		0.069	0.208	**SIG**
Stage (S)		0.049	0.147	**SIG**
Interaction (TxS)		0.085	0.254	**NS**

* In 45 DAS the grain was not developed so the observation was taken at 75 DAS only to estimate the nutrient (N, P, K, S and Cd) content in the mustard grain.

The effects of treatment showed significant effect on Cd uptake in the mustard grain which was found 0.57 ppm (T3) and 0.47 ppm (T2) respectively (Table 5.6 and Appendix 1.6). The T4 (RDF+AM+SSP) was found most prominent effect Cd uptake in the grain (0.60 ppm) where as T1 (RDF) was found in the least content (0.27 ppm) in the mustard grain. The stages of the crop growth had significant effect of Cd uptake on the grain. In 90 DAS (0.60 ppm) the grain had much higher Cd uptake than the 75 DAS (0.35 ppm). There was no significant role which the interaction growing stage and treatment in the Cd uptake of in mustard grain.

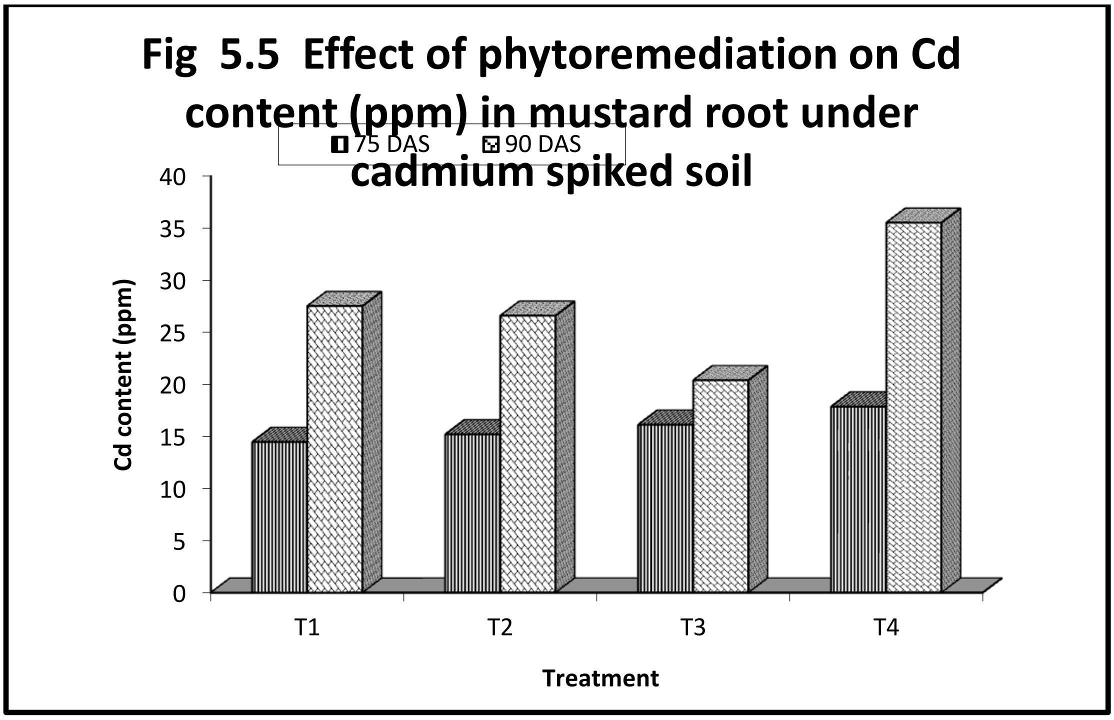


Fig 5.5 Effect of phytoremediation on Cd content (ppm) in mustard root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

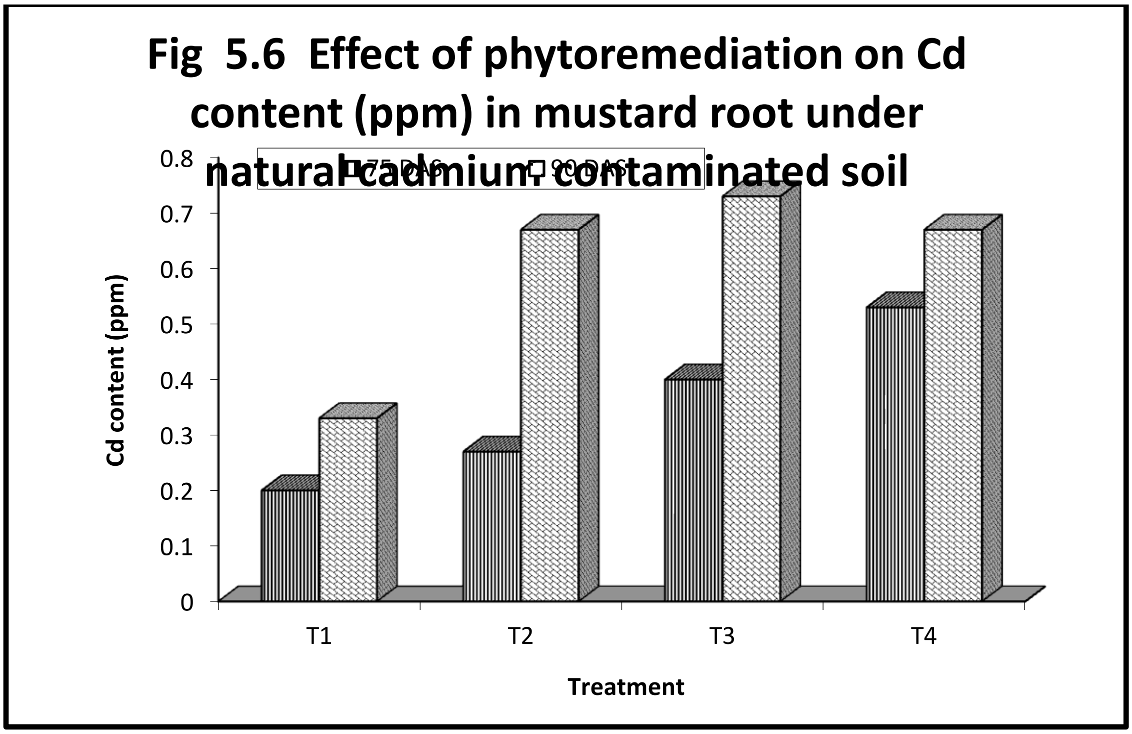


Fig 5.6 Effect of phytoremediation on Cd content (ppm) in mustard root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 6.1 Effect of phytoremediation on soil available N (kg ha^{-1}) in mustard crop under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	117.1	66.1	91.6
T2	**RDF+SSP**	133.8	76.9	105.4
T3	**RDF+AM**	142.2	88.6	115.4
T4	**RDF+SSP+AM**	154.7	104.1	129.4
Mean		137.0	83.9	
		SEm±	**CD at 5% level**	
Treatment (T)		13.26	39.77	**NS**
Stage (S)		9.38	28.12	**SIG**
Interaction (TxS)		16.25	48.70	**NS**

In Cd spiked soil, the effects of treatment had non-significant effect on soil available N under mustard crop (Table, Fig 6.1 and Appendix 1.7). The stages of the crop growth had significant effect on soil available N. In 45 DAS (137 kg ha^{-1}) the mustard had much higher available N than the 90 DAS (83.9 kg ha^{-1}). The interaction effect between growing stage and treatment had non-significant in the available N in mustard crop.

Table 6.2 Effect of phytoremediation on soil available N (kg ha^{-1}) in mustard crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	118.7	76.9	97.8
T2	**RDF+SSP**	154.7	88.2	121.5
T3	**RDF+AM**	175.6	96.2	135.9
T4	**RDF+SSP+AM**	192.3	102.0	147.2
Mean		160.3	90.8	
		SEm±	**CD at 5% level**	
Treatment (T)		17.96	53.84	**NS**
Stage (S)		12.70	38.07	**SIG**
Interaction (TxS)		21.99	65.93	**NS**

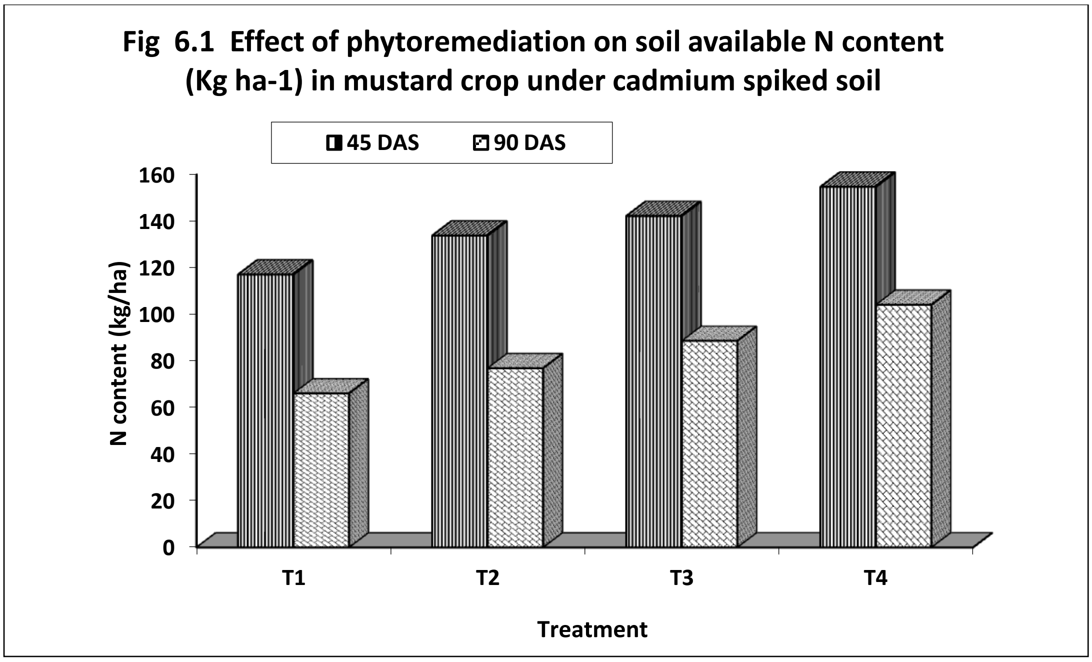


Fig 6.1 Effect of phytoremediation on soil available N content (Kg ha-1) in mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

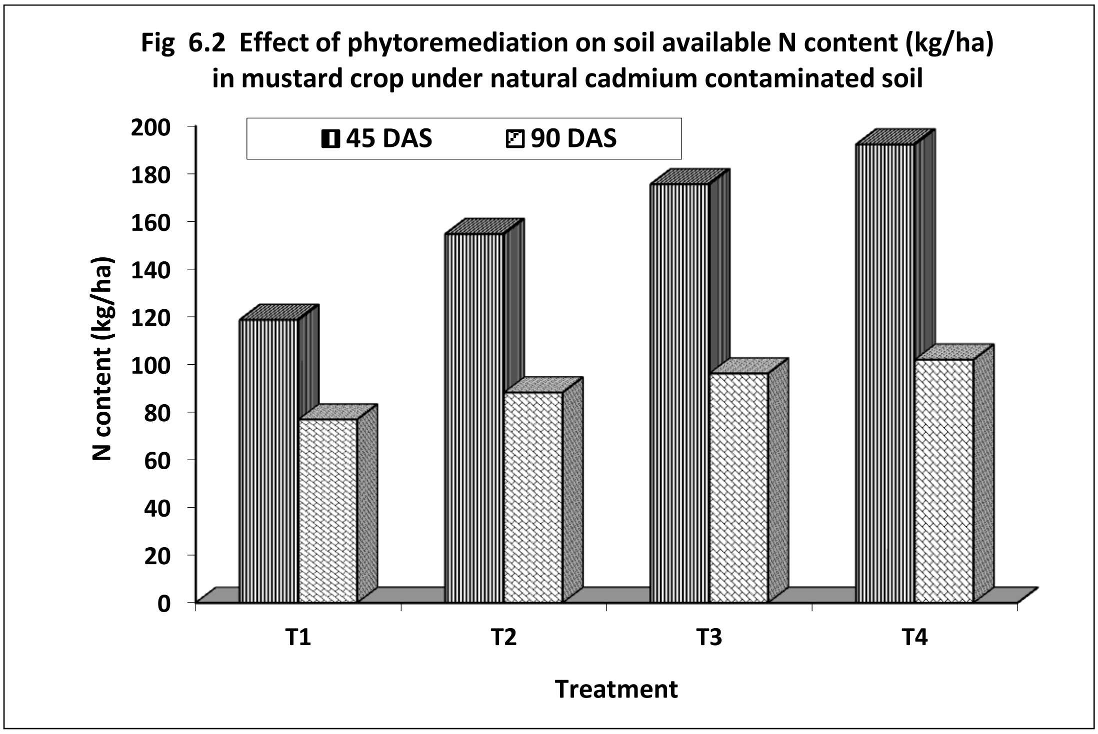


Fig 6.2 Effect of phytoremediation on soil available N content (kg/ha) in mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

In naturally Cd contaminated soil, the effects of treatment had significant effect on available N in the mustard crop which was observed 135.9 kg ha^{-1} (T3) and 121.9 kg ha^{-1} (T2) respectively (Table, Fig 6.2 and Appendix 1.8). The T4 (RDF+AM+SSP) was found most prominent effect available N (147.2 kg ha^{-1}) whereas in T1 (RDF) was showed in the least content (97.8 kg ha^{-1}) in the mustard crop. The stages of the crop growth had significant effect on soil available N. In 45 DAS (160.3 kg ha^{-1}) the mustard had much the higher soil available N than the 90 DAS (90.8 kg ha^{-1}). The interaction effect between growing stage and treatment was found non-significant.

Table 7.1 Effect of phytoremediation on soil available P (kg ha^{-1}) in mustard crop under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	70.3	76.2	73.2
T2	**RDF+SSP**	85.9	84.5	85.2
T3	**RDF+AM**	89.5	96.4	93.0
T4	**RDF+SSP+AM**	97.4	106.5	101.9
Mean		85.8	90.9	
		SEm±	**CD at 5% level**	
Treatment (T)		1.627	4.876	**SIG**
Stage (S)		1.150	3.448	**SIG**
Interaction (TxS)		1.992	5.972	**NS**

In Cd spiked soil, the effects of treatment had significant effect on soil available P in the mustard which was found 93.0 kg ha^{-1} (T3) and 85.2 kg ha^{-1} (T2) respectively (Table, Fig 7.1 and Appendix 1.7). The T4 (RDF+AM + SSP) was found most prominent effect on soil available P (101.9 kg ha^{-1}) where as is T1 (RDF) was observed in the least available P (73.2 kg ha^{-1}) in the mustard. The stages of the crop growth had significant effect on available P on the mustard crop. In 90 DAS (90.9 kg ha^{-1}) the mustard had much the higher available P than the 45 DAS (85.8 kg ha^{-1}). The interaction effect between growing stage and treatment had non-significant in the available P in mustard crop.

Table 7.2 Effect of phytoremediation on soil available P (kg ha^{-1}) in mustard crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	89.1	89.1	89.1
T2	**RDF+SSP**	95.5	106.5	101.0
T3	**RDF+AM**	100.1	114.3	107.2
T4	**RDF+SSP+AM**	103.3	131.3	117.3
Mean		97.0	110.3	
		SEm±	**CD at 5% level**	
Treatment (T)		4.16	12.48	**SIG**
Stage (S)		2.94	8.82	**SIG**
Interaction (TxS)		5.10	15.28	**NS**

The effects of treatment showed significant effect on available P in the mustard crop which was observed 107.2 kg ha^{-1} (T3) and 101.2 kg ha^{-1} (T2) respectively (Table, Fig 7.2 and Appendix 1.8). The T4 (RDF+AM+SSP) was found most prominent effect available P (117.3 kg ha^{-1}) where as T1 (RDF) was found in the least content (89.1 kg ha^{-1}) in the mustard crop. The stages of the crop growth had significant effect on available P on the spinach crop. In 90 DAS (110.3 kg ha^{-1}) the mustard had much the higher available P than the 45 DAS (97.0 kg ha^{-1}). The interaction effect between growing stage and treatment was found non-significant.

Table 8.1 Effect of phytoremediation on soil available K (kg ha^{-1}) in mustard crop under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	339.7	310.2	325.0
T2	**RDF+SSP**	351.3	327.0	339.2
T3	**RDF+AM**	357.3	345.3	351.3
T4	**RDF+SSP+AM**	369.2	358.4	363.8
Mean		354.4	335.2	
		SEm±	**CD at 5% level**	
Treatment (T)		5.18	15.54	**SIG**
Stage (S)		3.66	10.99	**SIG**
Interaction (TxS)		6.35	19.03	**NS**

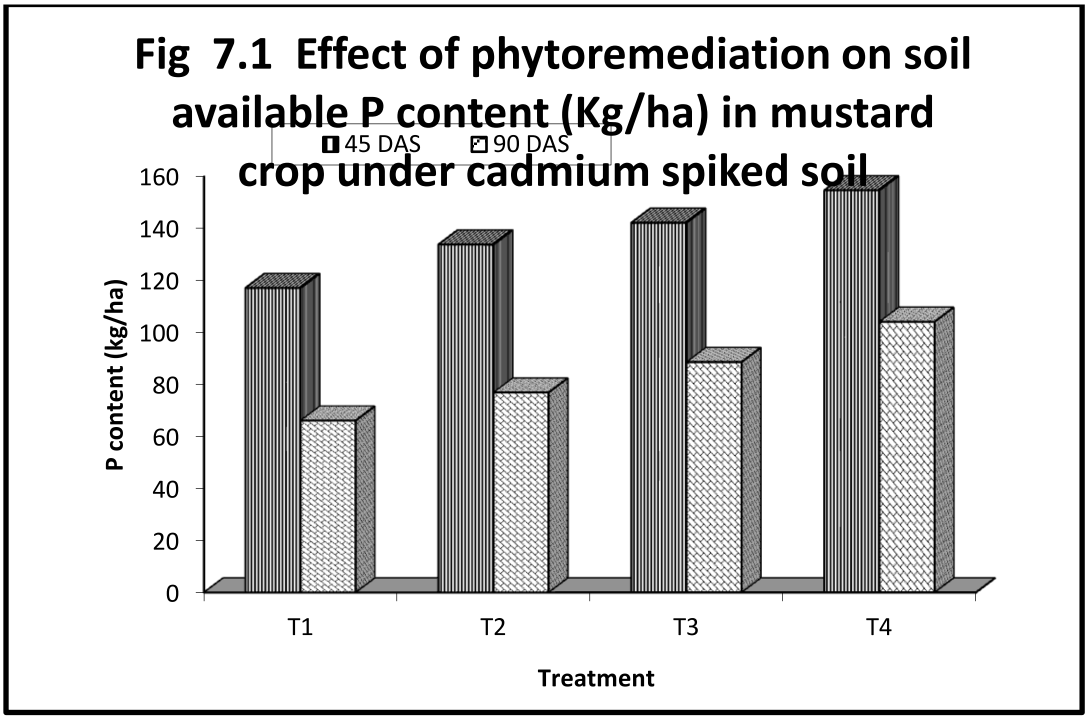


Fig 7.1 Effect of phytoremediation on soil available P content (Kg/ha) in mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

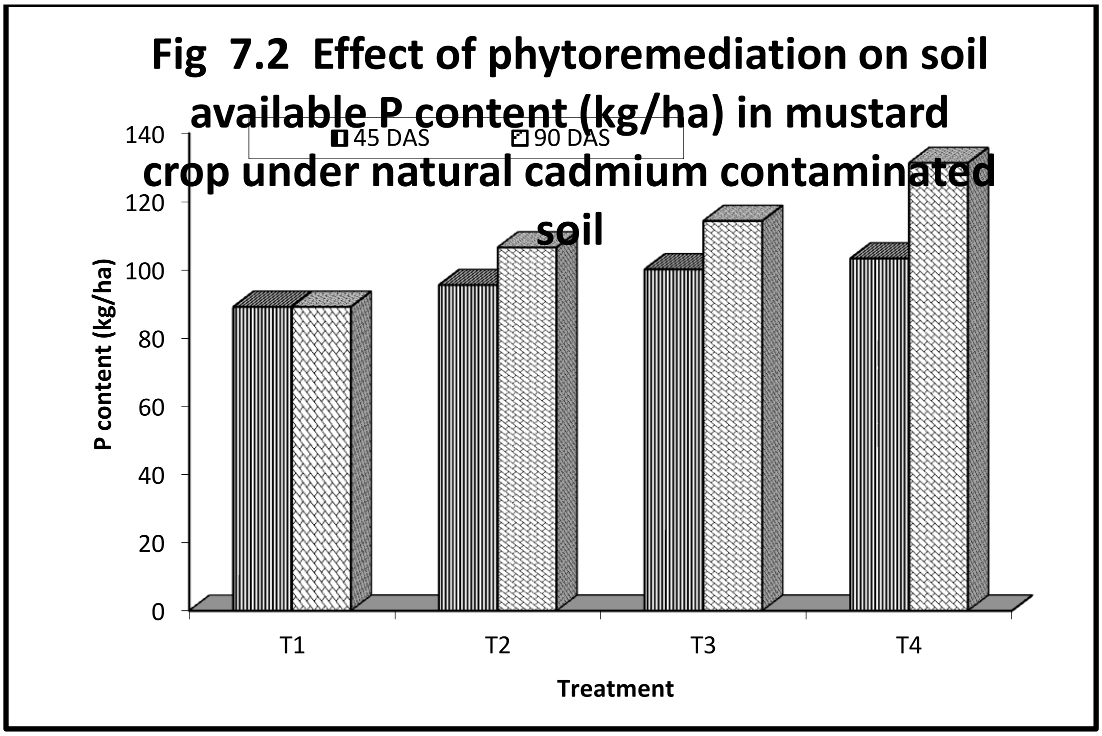


Fig 7.2 Effect of phytoremediation on soil available P content (kg/ha) in mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

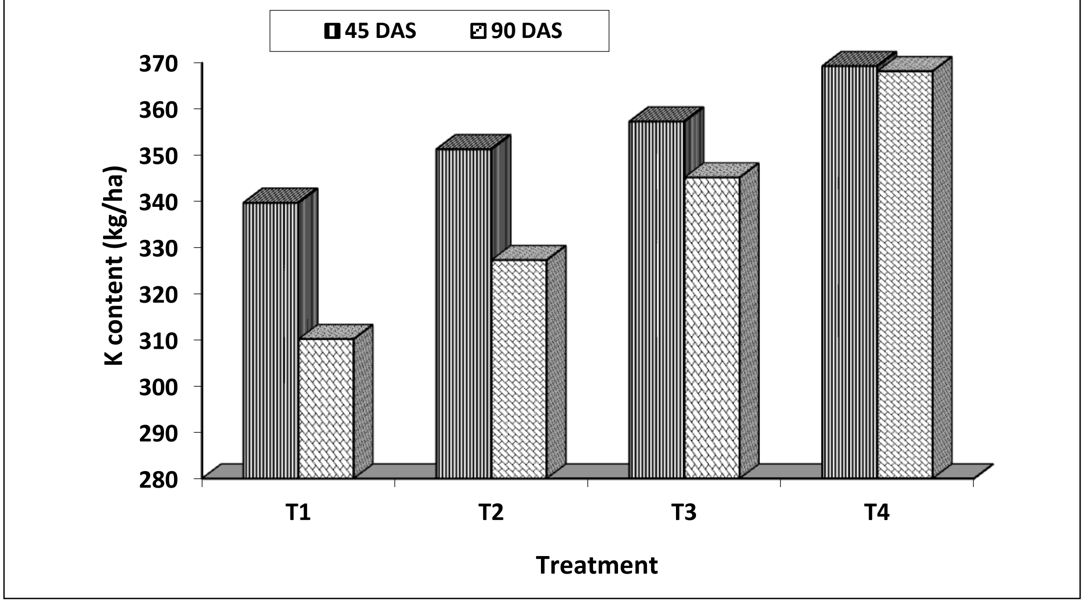
In Cd spiked soil, the effects of treatment had significant effect on available K in the mustard which was found 351.3 kg ha^{-1} (T3) and 339.2 kg ha^{-1} (T2) respectively (Table ,Fig 8.1 and Appendix 1.8). The treatment i.e. T4 (RDF+AM+SSP) was found most prominent effect available K (363.8 kg ha^{-1}) whereas in T1 (RDF) was found in the least available K (325.0 kg ha^{-1}) in the mustard. The stages of the crop growth had significant effect on available K on the mustard crop. In 45 DAS (354.4 kg ha^{-1}) the mustard had much the higher available K than the 90 DAS (335.2 kg ha^{-1}). The interaction effect between growing stage and treatments had non-significant in the available K in mustard crop.

Table 8.2 Effect of phytoremediation on soil available K (kg ha^{-1}) in mustard crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	346.8	345.3	346.1
T2	**RDF+SSP**	368.9	350.6	359.7
T3	**RDF+AM**	381.5	357.7	369.6
T4	**RDF+SSP+AM**	391.6	370.7	381.2
Mean		372.2	356.1	
		SEm±	**CD at 5% level**	
Treatment (T)		4.58	13.74	**SIG**
Stage (S)		3.24	9.72	**SIG**
Interaction (TxS)		5.61	16.83	**NS**

In naturally Cd contaminated soil, the effects of treatment had significant effect on available K in the mustard crop which was observed 369.6 kg ha^{-1} (T3) and 359.7 kg ha^{-1} (T2) respectively (Table, Fig 8.2 and Appendix 1.8). The T4 (RDF+AM+SSP) was found most prominent effect available K (381.2 kg ha^{-1}) where as T1 (RDF) was showed in the least content (346.1 kg ha^{-1}) in the mustard crop. The stages of the crop growth had significant effect on available K on the spinach crop. In 45 DAS (372.2 kg ha^{-1}) the mustard had much the higher available K than the 90 DAS (356.1 kg ha^{-1}). The interaction effect between growing stage and treatment was found non-significant.

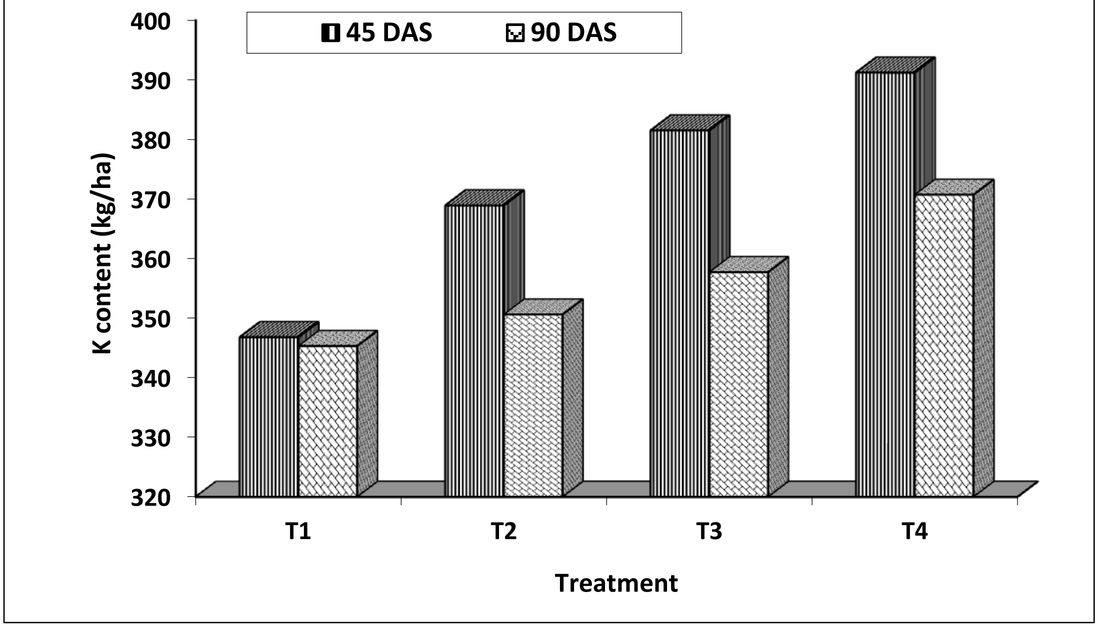
Fig 8.1 Effect of phytoremediation on soil available K content (Kg/ha) in mustard crop under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 8.2 Effect of phytoremediation on soil available K content (kg/ha) in mustard crop under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 9.1 Effect of phytoremediation on soil available S (mg kg^{-1}) in mustard crop under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.039	0.042	0.040
T2	**RDF+SSP**	0.041	0.045	0.043
T3	**RDF+AM**	0.046	0.043	0.045
T4	**RDF+SSP+AM**	0.051	0.044	0.048
Mean		0.044	0.074	
		SEm±	**CD at 5% level**	
Treatment (T)		0.015	0.045	**SIG**
Stage (S)		0.010	0.031	**NS**
Interaction (TxS)		0.018	0.055	**SIG**

The effects of treatment had significant effect on available S in the mustard which was found 0.045 mg kg^{-1} (T3) and 0.043 mg kg^{-1} (T2) respectively (Table 9.1 and Appendix 1.7). The T4 (RDF+AM+SSP) was showed most prominent effect available S 0.048 mg kg^{-1} whereas in T1 (RDF) it was found in the least available S (0.040 mg kg^{-1}) in the mustard. The stages of the crop growth had non-significant effect on available S on the mustard crop. In this study the interaction between growing stage of mustard and treatment had significant role on soil available S. In respect to interaction, the 45 DAS among treatment the T4 (0.051 mg kg^{-1}) was observed superior than other treatment combination with growing stage of mustard grain the S accumulation decrease and which was found highest in T4 treatment.

Table 9.2 Effect of phytoremediation on soil available S (mg kg^{-1}) in mustard crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (Mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.048	0.043	0.045
T2	**RDF+SSP**	0.050	0.047	0.048
T3	**RDF+AM**	0.053	0.052	0.052
T4	**RDF+SSP+AM**	0.057	0.058	0.057
Mean		0.052	0.050	
		SEm±	**CD at 5% level**	
Treatment (T)		0.001	0.002	**SIG**
Stage (S)		0.000	0.001	**SIG**
Interaction (TxS)		0.001	0.002	**NS**

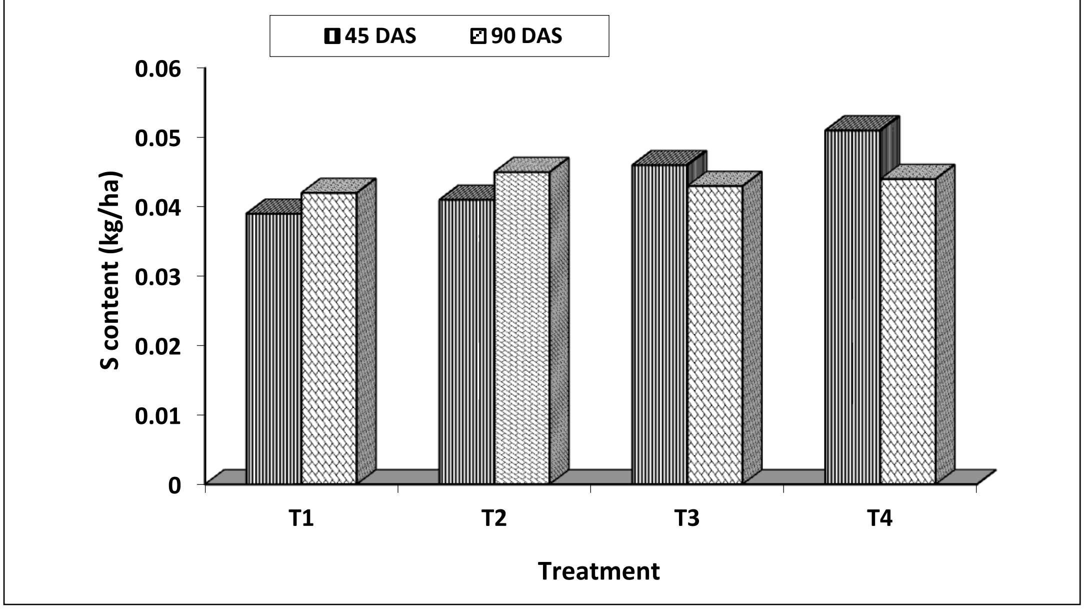


Fig 9.1 Effect of phytoremediation on available S content (Kg/ha) in mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

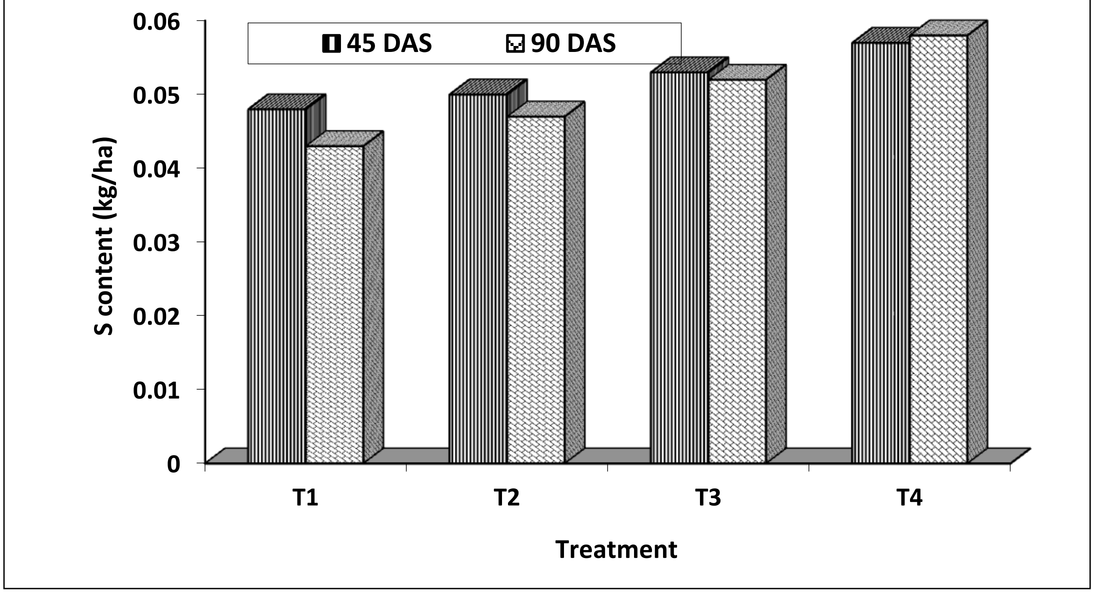


Fig 9.2 Effect of phytoremediation on available S content (kg/ha) in mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on available S in the mustard crop which was observed 0.052 mg kg^{-1} (T3) and 0.048 mg kg^{-1} (T2) respectively (Table, Fig 9.2 and Appendix 1.8). The T4 (RDF+AM+SSP) was found most prominent effect available S (0.057 mg kg^{-1}) whereas in T1 (RDF) was showed in the least content (0.045 mg kg^{-1}) in the mustard crop. The stages of the crop growth had significant effect on available S on the spinach crop. In 45 DAS (0.052 mg kg^{-1}) the mustard had much the higher available S than the 90 DAS (0.050 mg kg^{-1}). The interaction effect between growing stage and treatment was found non-significant.

Table 10.1 Effect of phytoremediation on soil Cd (ppm) in mustard crop under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	52.41	43.65	48.03
T2	**RDF+SSP**	61.65	57.07	59.36
T3	**RDF+AM**	72.49	67.41	69.95
T4	**RDF+SSP+AM**	56.55	47.78	52.17
Mean		60.77	53.98	
		SEm±	**CD at 5% level**	
Treatment (T)		1.77	5.31	**SIG**
Stage (S)		1.25	3.75	**SIG**
Interaction (TxS)		2.17	6.50	**NS**

In case of Cd spiked soil, the effects of treatment had significant effect on soil available Cd in mustard which was found 59.36 ppm (T2) and 52.17 ppm (T4) respectively (Table, Fig 10.1 and Appendix 1.7). The T3 (RDF+AM) was found most prominent effect soil available Cd 69.95 ppm whereas T1 (RDF) was observed in the least available Cd (48.03 ppm) in the mustard. The stages of the crop growth had significant effect on available Cd on the mustard crop. In 45 DAS (60.77 ppm) the mustard had much the higher available Cd than the 90 DAS (53.58 ppm). The interaction effect between growing stage and treatment was found non-significant.

Table 10.2 Effect of phytoremediation on soil Cd (ppm) in mustard crop under naturally cadmium contaminated soil

	Stage of plant growth (mustard)

Treatment		**45 DAS**	**90 DAS**	**Mean**
T1	**RDF**	0.42	0.33	0.38
T2	**RDF+SSP**	0.51	0.38	0.45
T3	**RDF+AM**	0.60	0.42	0.51
T4	**RDF+SSP+AM**	0.46	0.36	0.41
Mean		0.50	0.37	
		SEm±	**CD at 5% level**	
Treatment (T)		0.033	0.098	**NS**
Stage (S)		0.023	0.069	**SIG**
Interaction (TxS)		0.040	0.119	**NS**

Under Cd spiked soil, the effects of treatment had non-significant effect on available Cd during the mustard crop (Table, Fig 10.2 and Appendix 1.8). The stages of the crop growth have significant effect on soil available Cd on the spinach crop. During the growing period of mustard, it was observed that in 45 DAS (0.50 ppm) soil Cd concentration was found significantly higher as compared advancement of the growing period i.e. 90 DAS (0.37 ppm).

Table 11.1 Effect of phytoremediation on dehydrogenase activity (*µg* TPF g^{-1} soil 24h^{-1}) in mustard crop under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	31	72	51.27
T2	**RDF+SSP**	44	77	60.15
T3	**RDF+AM**	54	84	69.02
T4	**RDF+SSP+AM**	63	94	78.88
Mean		48	82	
		SEm±	**CD at 5% level**	
Treatment (T)		8.57	25.70	**NS**
Stage (S)		6.06	18.17	**SIG**
Interaction (TxS)		10.50	31.48	**NS**

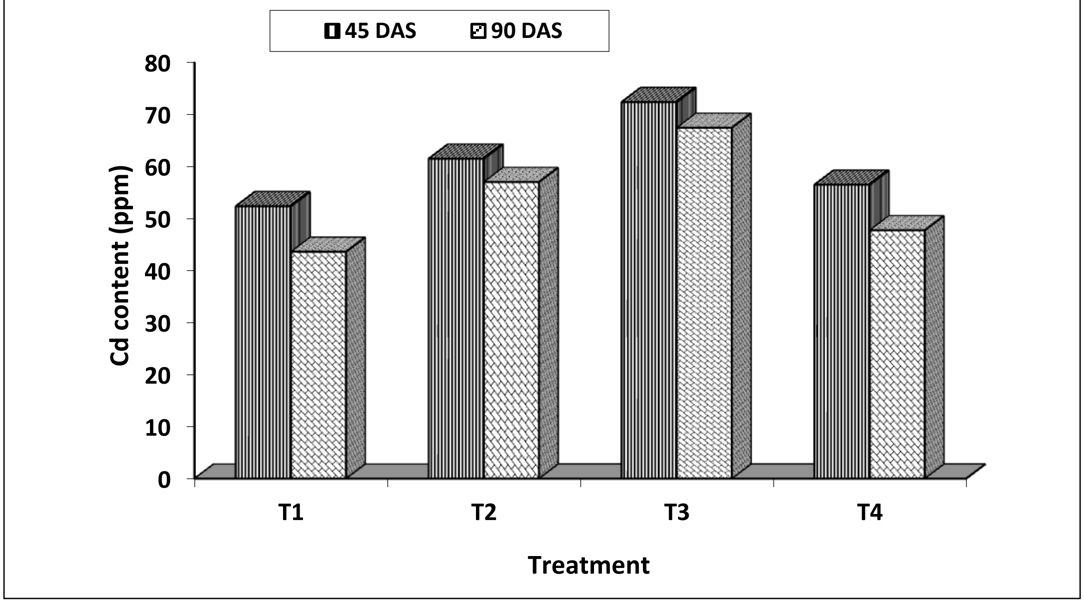


Fig 10.1 Effect of phytoremediation on available Cd content (ppm) in mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

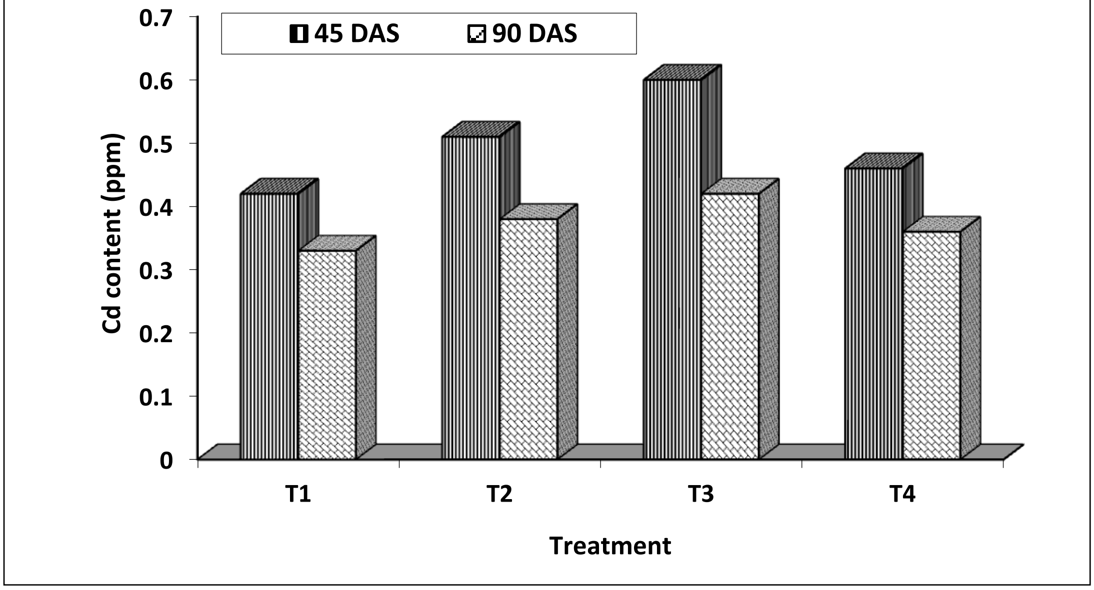


Fig 10.2 Effect of phytoremediation on available Cd content (ppm) in mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on dehydrogenase activity in the mustard crop (Table, Fig 11.1 and Appendix 1.9). The stages of the crop growth had significant effect on soil dehydrogenase activity on the mustard crop. In 90 DAS (82 µg TPF g^{-1} soil 24 h^{-1}) the mustard had much the higher soil dehydrogenase activity than the 45 DAS (48 µg TPF g^{-1} soil $24h^{-1}$). The interaction effects between growing stage and treatment was found no role in the DHA in mustard crop.

Table 11.2 Effect of phytoremediation on dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$) during mustard crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	27	134	80
T2	**RDF+SSP**	55	146	100
T3	**RDF+AM**	59	153	106
T4	**RDF+SSP+AM**	73	178	125
Mean		53	153	
		SEm±	**CD at 5% level**	
Treatment (T)		24.88	74.58	**NS**
Stage (S)		17.59	52.73	**SIG**
Interaction (TxS)		30.47	91.34	**NS**

In naturally Cd contaminated soil, the effects of treatment had non-significant effect on soil dehydrogenase activity in the mustard crop (Table, Fig 11.2 and Appendix 1.10). The stages of the crop growth had significant effect on dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1)}$ on the mustard crop. In 90 DAS (153 µg TPF g^{-1} soil $24h^{-1}$) the mustard had much the higher dehydrogenase activity than the 45 DAS (53 µg TPF g^{-1} soil $24h^{-1}$). The interaction effect between growing stage and treatment was found in non-significant.

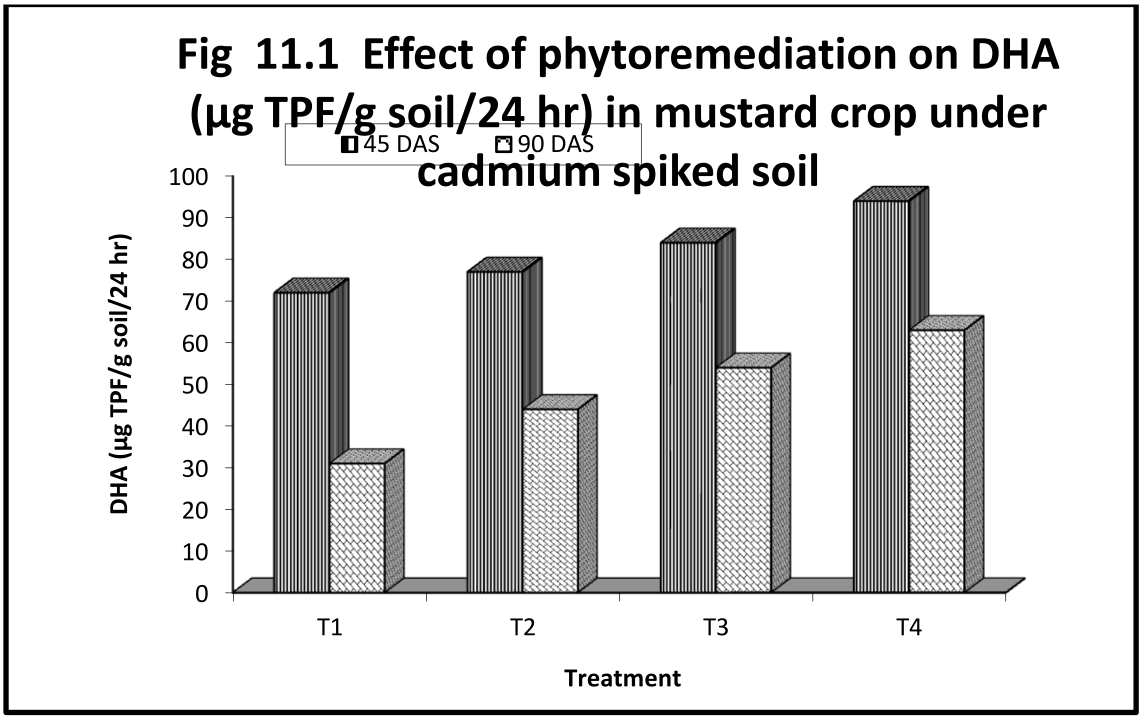


Fig 11.1 Effect of phytoremediation on DHA (µg TPF/g soil/24 hr) in mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

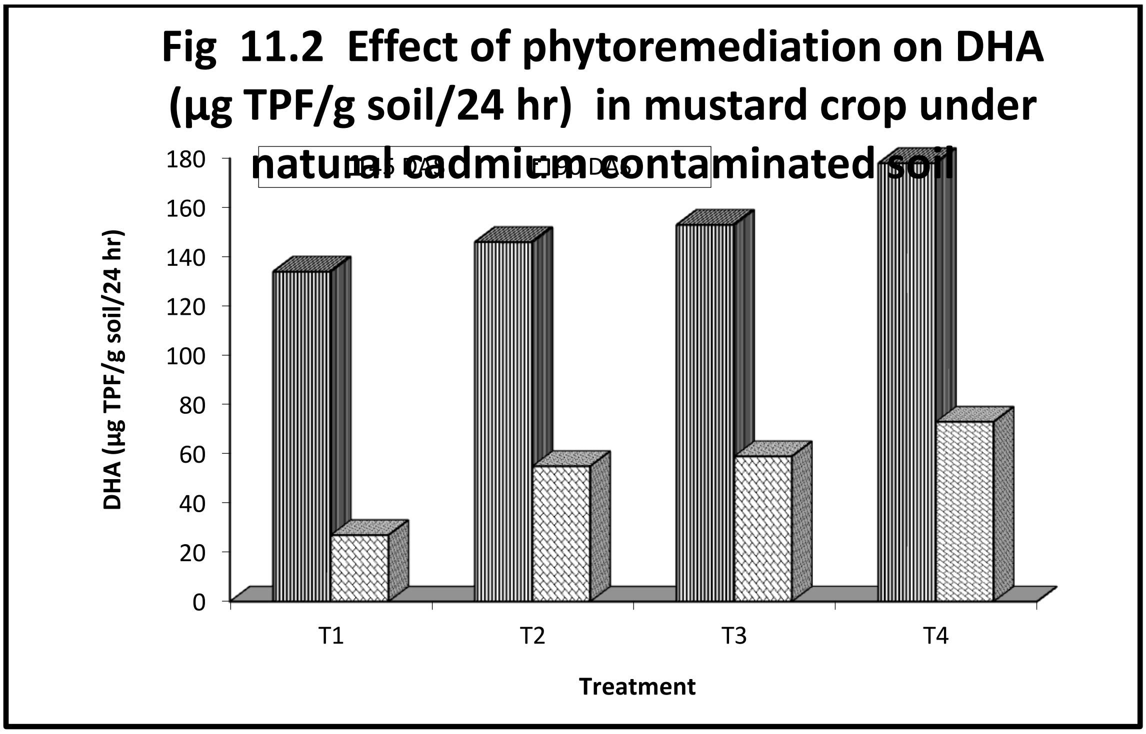


Fig 11.2 Effect of phytoremediation on DHA (µg TPF/g soil/24 hr) in mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 12.1 Effect of phytoremediation on fluorescein di-acetate (*µg* fluorescein g^{-1}) in mustard crop under cadmium spiked soil

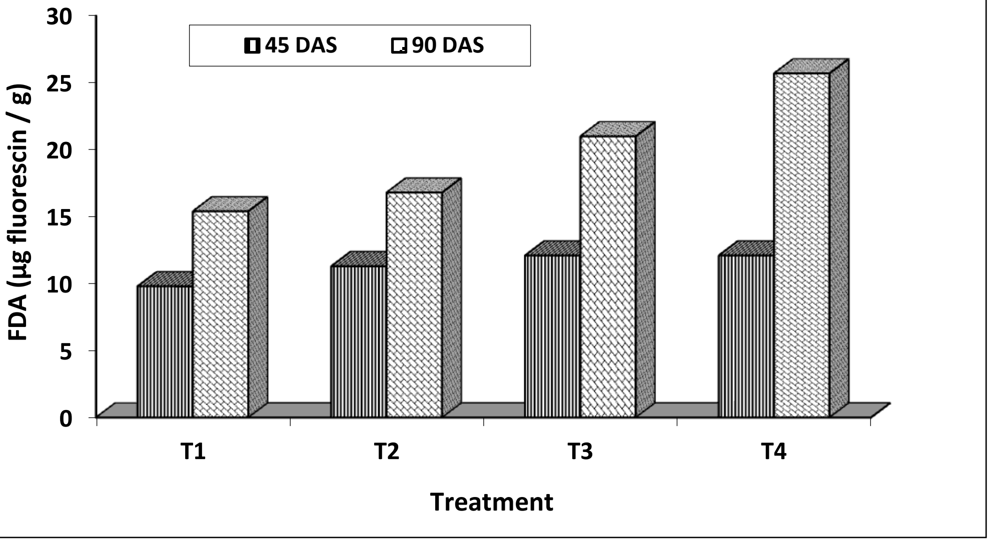
Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	9.8	15.4	12.6
T2	**RDF+SSP**	11.3	18.8	15.1
T3	**RDF+AM**	12.1	21.0	16.6
T4	**RDF+SSP+AM**	12.1	25.7	18.9
Mean		11.3	20.2	
		SEm±	**CD at 5% level**	
Treatment (T)		2.34	7.03	**NS**
Stage (S)		1.66	4.97	**SIG**
Interaction (TxS)		2.87	8.60	**NS**

In the Cd spiked soil, the effects of treatment had non-significant effect on fluorescein di-acetate (FDA) in the mustard (Table, Fig 12.1 and Appendix 1.19). The stages of the crop growth had significant effect on available FDA on the mustard crop. In 90 DAS (20.2 µg fluorescein g^{-1}) the mustard had much the higher FDA than the 45 DAS (11.3 µg fluorescein g^{-1}). The interaction effect between growing stage and treatment have non-significant in the FDA in mustard crop.

Table 12.2 Effect of phytoremediation on fluorescein di-acetate (*µg* fluorescein g^{-1}) activity in mustard crop under naturally Cd contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	13.3	17.3	15.3
T2	**RDF+SSP**	15.1	17.2	16.1
T3	**RDF+AM**	16.8	20.7	18.8
T4	**RDF+SSP+AM**	18.2	22.7	20.4
Mean		15.8	19.5	
		SEm±	**CD at 5% level**	
Treatment (T)		0.93	2.80	**SIG**
Stage (S)		0.66	1.98	**SIG**
Interaction (TxS)		1.14	3.43	**NS**

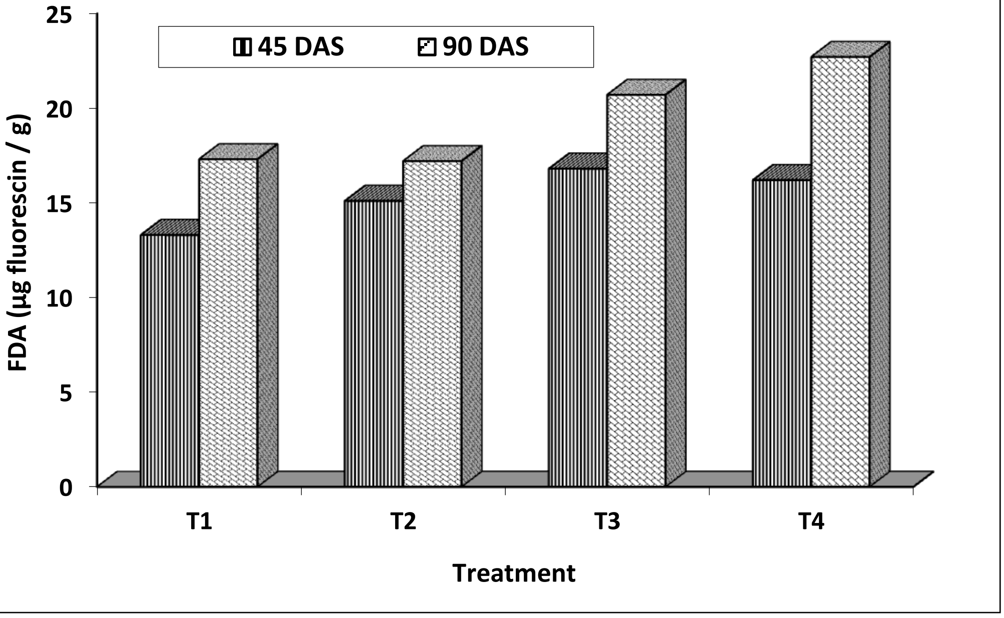
Fig 12.1 Effect of phytoremediation on fluorescein di-acetate (µg fluorescein g^{-1}) in mustard crop under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 12.2 Effect of phytoremediation on fluorescein di-acetate (µg fluorescein g^{-1}) activity in mustard crop under naturally Cd contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on fluorescein di-acetate (FDA) in the mustard crop which was observed 18.8 *µg* fluorescein g^{-1} (T3) and 16.1 *µg* fluorescein g^{-1} (T2) respectively (Table, Fig 12.2 (Table, Fig 12.2 and Appendix 1.20). The T4 (RDF+AM+SSP) was found most prominent effect available FDA (20.4 *µg* fluorescein g^{-1}) where as T1 (RDF) was found in the least content (15.3 µg fluorescein g^{-1}) in the mustard crop. The stages of the crop growth had significant effect on FDA on the mustard crop. In 90 DAS (19.5 *µg* fluorescein g^{-1}) the mustard had much the higher FDA than the 45 DAS (15.8 *µg* fluorescein g^{-1}). The interaction effect between growing stage and treatment was found non-significant.

Table 13.1 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in mustard crop under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.038	0.041	0.037
T2	**RDF+SSP**	0.044	0.045	0.044
T3	**RDF+AM**	0.052	0.059	0.056
T4	**RDF+SSP+AM**	0.072	0.079	0.070
Mean		0.052	0.056	
		SEm±	**CD at 5% level**	
Treatment (T)		0.001	0.003	**SIG**
Stage (S)		0.001	0.002	**NS**
Interaction (TxS)		0.001	0.004	**SIG**

The effects of treatment had significant effect on soil respiration in the mustard which was found 0.056 mg CO_2-C g^{-1} soil d^{-1} (T3) and 0.044 mg CO_2-C g^{-1} soil d^{-1} (T2) respectively (Table, Fig 13.1 and Appendix 1.9). The T4 (RDF+AM+SSP) was found most prominent effect soil respiration 0.070 mg CO_2-C g^{-1} soil d^{-1} where as T1 (RDF) was found in the least soil respiration (0.037 mg CO_2-C g^{-1} soil d^{-1}) in the mustard. The stages of the crop growth had non-significant effect on soil respiration on the mustard crop. In this study the interaction between growing stage and treatment due to soil respiration in AM addition had significant role on mustard crop soil respiration in naturally cadmium contaminated. In 90 DAS among treatment the T4 (0.078 mg CO_2-C g^{-1} soil d^{-1}) was found superior than other treatment combination with growing stage of mustard the soil respiration decrease and which is observed highest in T4 treatment.

Table 13.2 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in mustard crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.034	0.041	0.038
T2	**RDF+SSP**	0.039	0.060	0.050
T3	**RDF+AM**	0.047	0.073	0.060
T4	**RDF+SSP+AM**	0.055	0.078	0.066
Mean		0.044	0.063	
		SEm±	**CD at 5% level**	
Treatment (T)		0.005	0.015	**SIG**
Stage (S)		0.004	0.011	**SIG**
Interaction (TxS)		0.006	0.019	**NS**

The effects of treatment have significant effect on soil respiration in the mustard crop which was observed 0.060 mg CO_2-C g^{-1} soil d^{-1} (T3) and 0.050 mg CO_2-C g^{-1} soil d^{-1} (T2) respectively (Table & Fig 13.2 and Appendix 1.10). The T4 (RDF+AM+SSP) was found most prominent effect soil respiration (0.066 mg CO_2-C g^{-1} soil d^{-1}) where as T1 (RDF) was found in the least content (0.038 mg CO_2-C g^{-1} soil d^{-1}) in the mustard crop. The stages of the crop growth had significant effect on soil respiration on the mustard crop. In 90 DAS (0.063 mg CO_2-C g^{-1} soil d^{-1}) the mustard had much the higher soil respiration than the 45 DAS (0.044 mg CO_2-C g^{-1} soil d^{-1}). The interaction effect between growing stage and treatment was found non-significant.

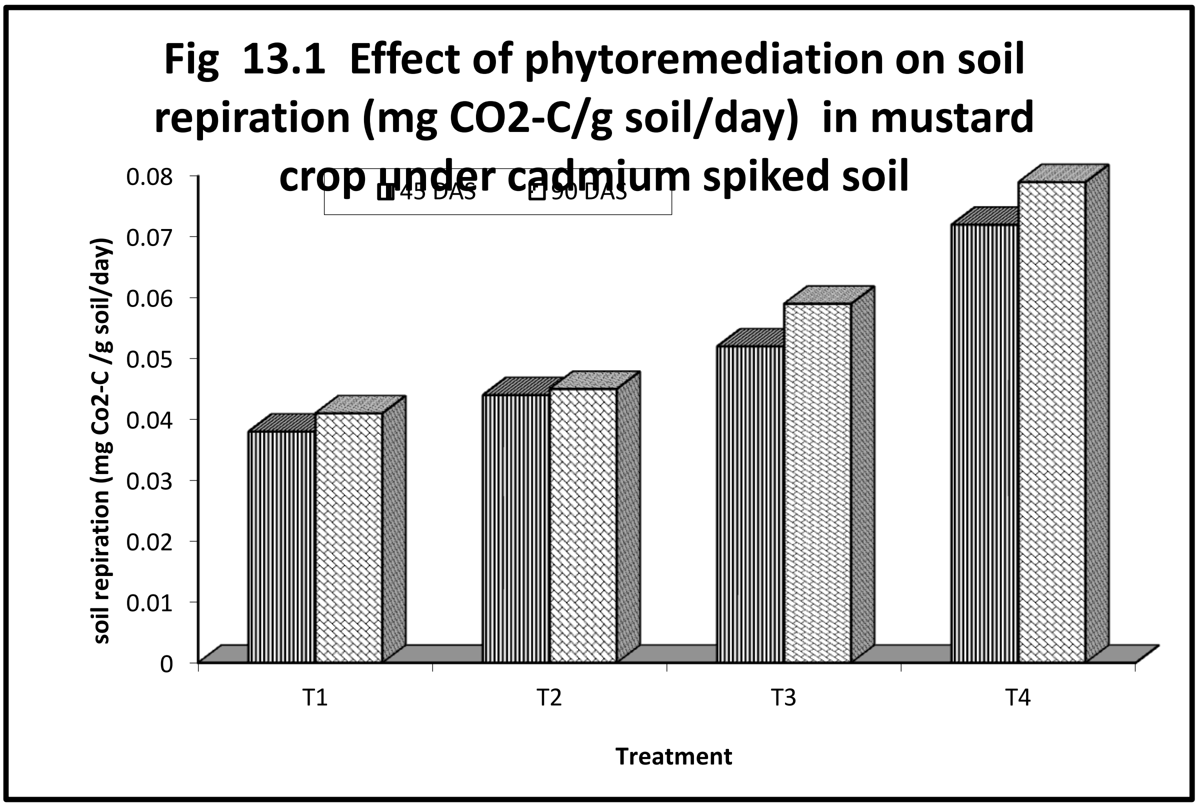


Fig 13.1 Effect of phytoremediation on soil repiration (mg CO2-C/g soil/day) in mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

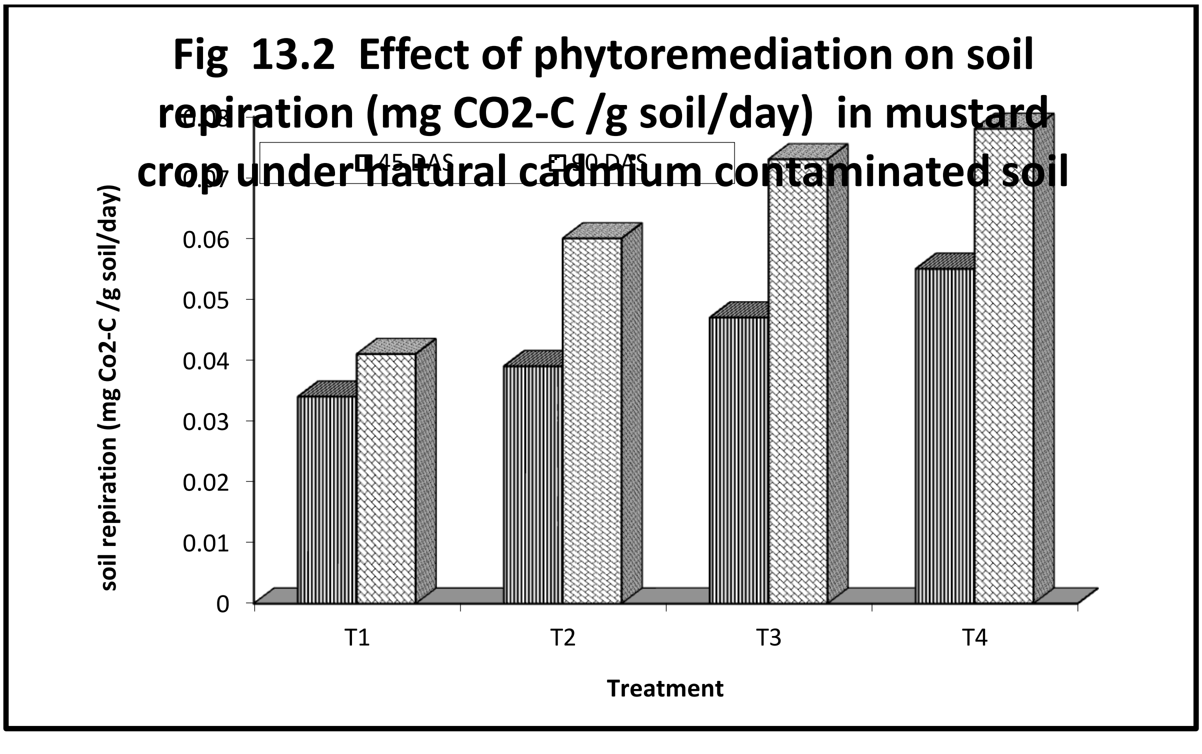


Fig 13.2 Effect of phytoremediation on soil repiration (mg CO2-C /g soil/day) in mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 14.1 Effect of phytoremediation on soil microbial biomass carbon (SMBC) in mustard crop under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	90 DAS	Mean
T1	**RDF**	0.59	0.68	0.64
T2	**RDF+SSP**	0.62	0.87	0.75
T3	**RDF+AM**	0.64	1.05	0.85
T4	**RDF+SSP+AM**	0.69	1.26	0.98
Mean		0.64	0.97	
		SEm	**CD at % level**	
Treatment (T)		0.094	0.282	**NS**
Stage (S)		0.066	0.199	**SIG**
Interaction (TxS)		0.115	0.345	**NS**

The effects of treatment had non-significant effect on SMBC in the mustard (Table, Fig 14.1 and Appendix 1.9). The stages of the crop growth have significant effect of SMBC on the mustard crop. In relation to growing stage of the crop, in 90 DAS (0.97) the mustard had much the higher SMBC than the 45 DAS (0.64). The interaction effects between growing stage and treatment was showed no role in the SMBC in mustard crop.

Table 14.2 Effect of phytoremediation on soil microbial biomass carbon (SMBC) in mustard under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	90 DAS	Mean
T1	**RDF**	0.86	1.26	1.06
T2	**RDF+SSP**	0.88	1.25	1.07
T3	**RDF+AM**	0.90	1.39	1.14
T4	**RDF+SSP+AM**	0.93	1.33	1.13
Mean		0.89	1.31	
		SEm±	**CD at 5% level**	
Treatment (T)		0.104	0.313	**NS**
Stage (S)		0.074	0.221	**SIG**
Interaction (TxS)		0.128	0.383	**NS**

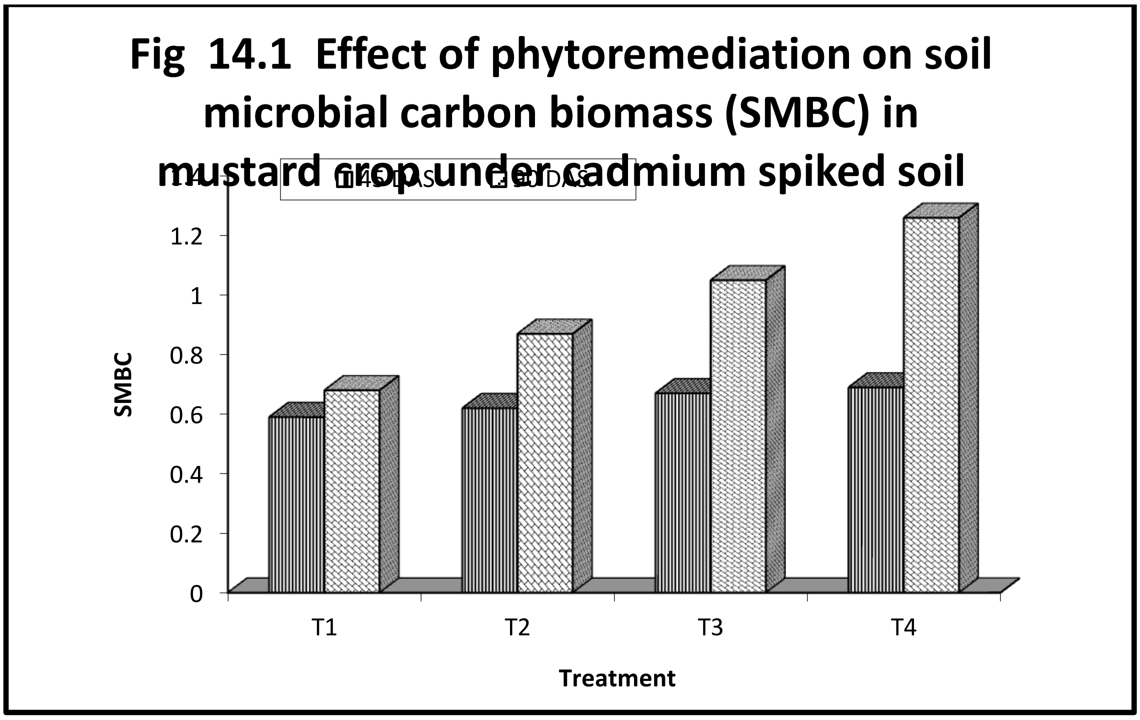


Fig 14.1 Effect of phytoremediation on soil microbial carbon biomass (SMBC) in mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

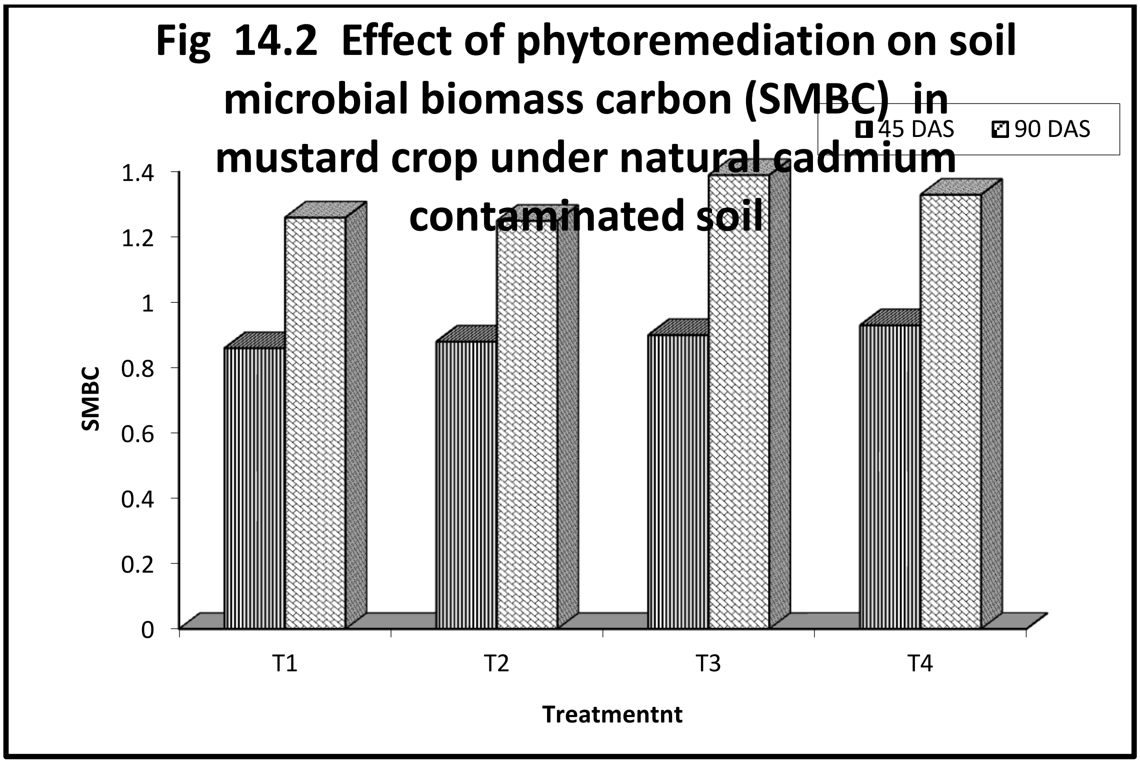


Fig 14.2 Effect of phytoremediation on soil microbial biomass carbon (SMBC) in mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on SMBC during the mustard crop (Table, Fig 14.2 and Appendix 1.10). The stages of the crop growth had significant effect of SMBC on the mustard crop. In 90 DAS (1.31) the mustard had much the higher SMBC than the 45 DAS (0.89). The interaction effect between growing stage and treatment was found in non-significant.

Table 15.1 Effect of phytoremediation on glomalin (mg kg^{-1}) content in soil during mustard crop under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	23.51	33.51	28.51
T2	**RDF+SSP**	27.14	34.04	30.59
T3	**RDF+AM**	28.16	34.92	31.54
T4	**RDF+SSP+AM**	32.67	42.42	37.54
Mean		27.87	36.22	
		SEm±	**CD at 5% level**	
Treatment (T)		2.12	6.36	**SIG**
Stage (S)		1.50	4.50	**SIG**
Interaction (TxS)		2.60	7.79	**NS**

The effects of treatment had significant effect on *glomalin* in the mustard crop which were observed 31.54 (mg kg^{-1}) (T3) and 30.59 (mg kg^{-1}) (T2) respectively (Table, Fig 15.1 and Appendix 1.9) whereasT4 (RDF+AM+ SSP) was found most prominent effect *glomalin* of (37.54 mg kg^{-1}) while T1 (RDF) was found in the least *glomalin* (28.51 mg kg^{-1}) in the mustard crop. The stages of the crop growth had significant effect of *glomalin* on the mustard crop. In 90 DAS (36.22 mg kg^{-1}) the mustard has much the higher available *glomalin* than the 45 DAS (27.87 mg kg^{-1}). The interaction effect between growing stage and treatment was observed in non-significant.

Table 15.2 Effect of phytoremediation on glomalin (mg kg^{-1}) content in soil during mustard crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	27.03	40.37	33.70
T2	**RDF+SSP**	34.57	43.82	39.20
T3	**RDF+AM**	35.48	44.32	39.90
T4	**RDF+SSP+AM**	40.62	52.80	46.71
Mean		34.43	45.33	
		SEm±	**CD at 5% level**	
Treatment (T)		2.77	8.30	**SIG**
Stage (S)		1.96	5.87	**SIG**
Interaction (TxS)		3.39	10.16	**NS**

The effects of treatment had significant effect on *glomalin* in the mustard crop which was observed 39.90 (mg kg^{-1}) (T3) and 39.20 (mg kg^{-1}) (T2) respectively (Table, Fig 15.2 and Appendix 1.10). The T4 (RDF+AM+ SSP) was found most prominent effect *glomalin* of (46.71 mg kg^{-1}) where as T1 (RDF) was observed in the least *glomalin* (33.70 mg kg^{-1}) in the mustard crop. The stages of the crop growth had significant effect of *glomalin* on the mustard crop. In 90 DAS (45.33 mg kg^{-1}) the mustard had much the higher available *glomalin* than the 45 DAS (34.43 mg kg^{-1}). The interaction effect between growing stage and treatment was observed in non-significant.

Table 16.1 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during mustard under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	2.11	1.67	1.89
T2	**RDF+SSP**	2.44	2.56	2.50
T3	**RDF+AM**	2.67	3.00	2.83
T4	**RDF+SSP+AM**	3.56	4.11	3.83
Mean		2.70	2.84	
		SEm±	**CD at 5% level**	
Treatment (T)		0.099	0.297	**SIG**
Stage (S)		0.070	0.210	**NS**
Interaction (TxS)		0.121	0.364	**SIG**

The effects of treatment had significant effect on fungi (cfu) in the mustard which was found in count of 2.83 cfu (T3) and 2.50 cfu (T2) respectively (Table, Fig 16.1 and

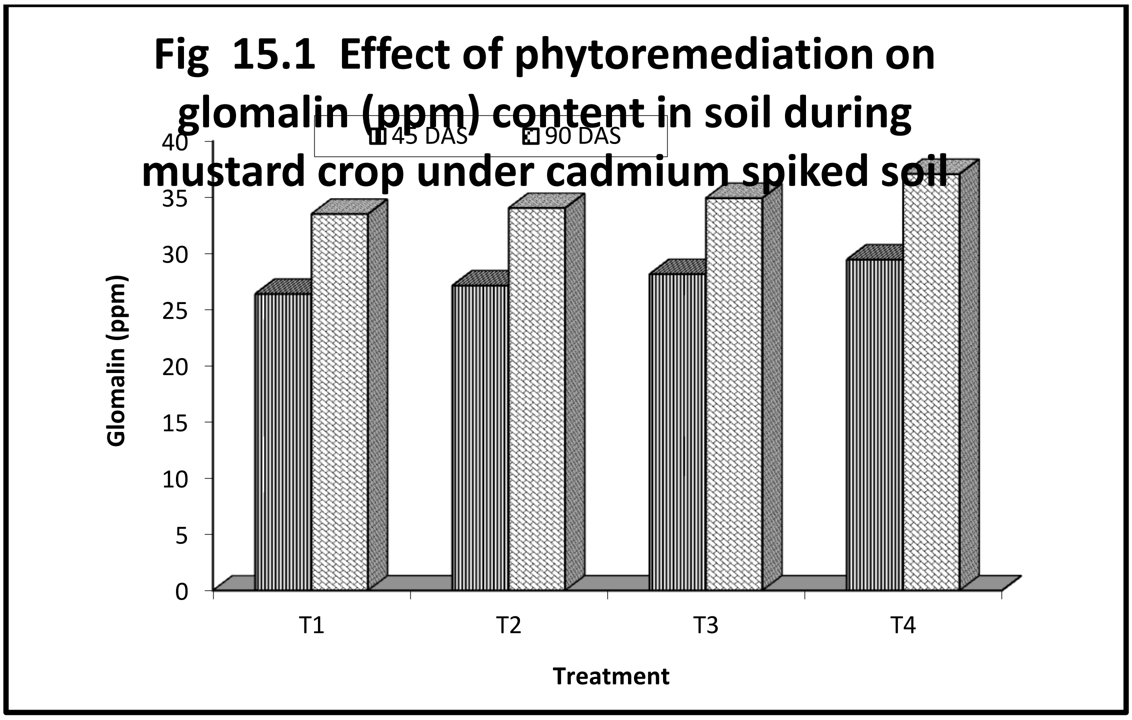


Fig 15.1 Effect of phytoremediation on glomalin (ppm) content in soil during mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

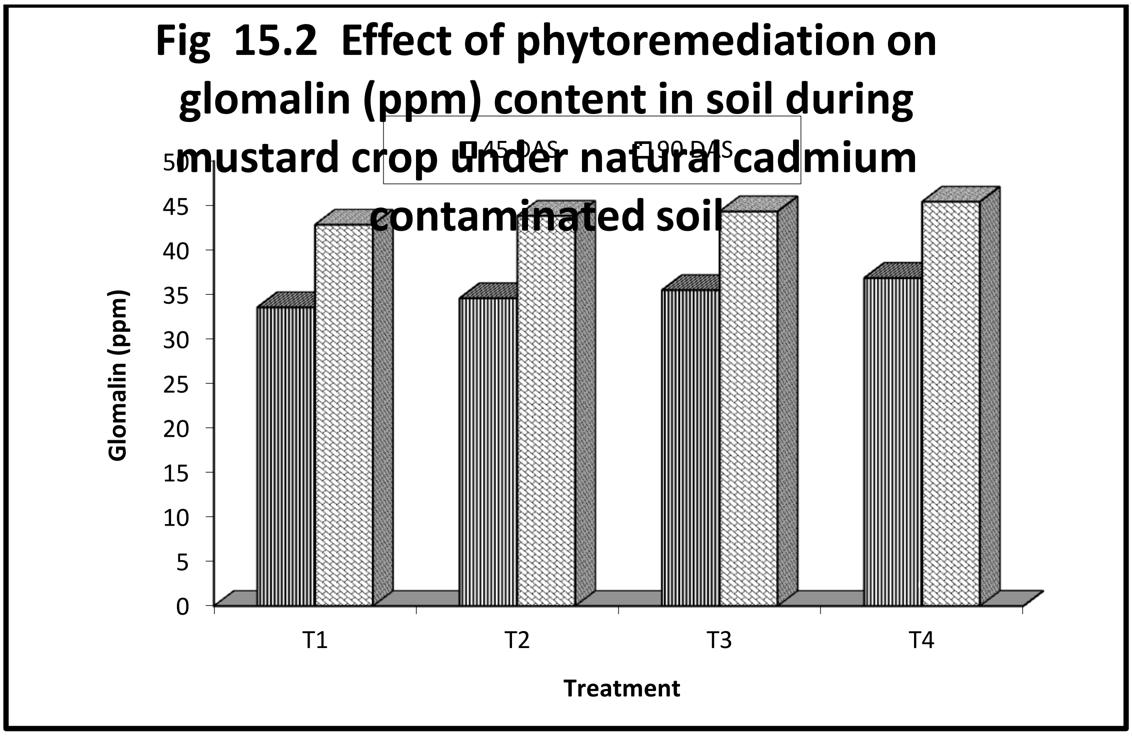


Fig 15.2 Effect of phytoremediation on glomalin (ppm) content in soil during mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Appendix 1.11). The T4 (RDF+AM+SSP) was found most prominent effect of fungi 3.83 (cfu) where as T1 (RDF) was observed in the least fungi in count of 1.89 (cfu) in the mustard crop. The stages of the crop growth had non-significant effect of fungi on the mustard crop. In this study the interaction between growing stage and treatment due to available fungi in AM addition had significant role on mustard crop fungi in cadmium spiked soil. In 90 DAS among treatment the T4 (4.11 cfu) was found superior than other treatment combination with growing stage of mustard the fungi decrease and which is showed highest in T4 treatment.

Table 16.2 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	2.33	2.44	2.39
T2	**RDF+SSP**	3.11	3.67	3.39
T3	**RDF+AM**	3.56	4.56	4.06
T4	**RDF+SSP+AM**	4.44	5.56	5.00
Mean		3.36	4.06	
		SEm±	**CD at 5% level**	
Treatment (T)		0.200	0.599	**SIG**
Stage (S)		0.141	0.424	**SIG**
Interaction (TxS)		0.245	0.734	**NS**

The effects of treatment had significant effect on fungi in the mustard crop which was observed count of 4.06 cfu (T3) and 3.39 cfu (T2) respectively (Table, Fig 16.2 and Appendix 1.12). The T4 (RDF+AM+SSP) was found most prominent effect available fungi count of (5.00 cfu) where as T1 (RDF) was found in the least available fungi count (2.39 cfu) in the mustard crop. The stages of the crop growth had significant effect of available fungi on the mustard crop. In 90 DAS (4.06 cfu) the mustard had much the higher available fungi than the 45 DAS (3.36 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

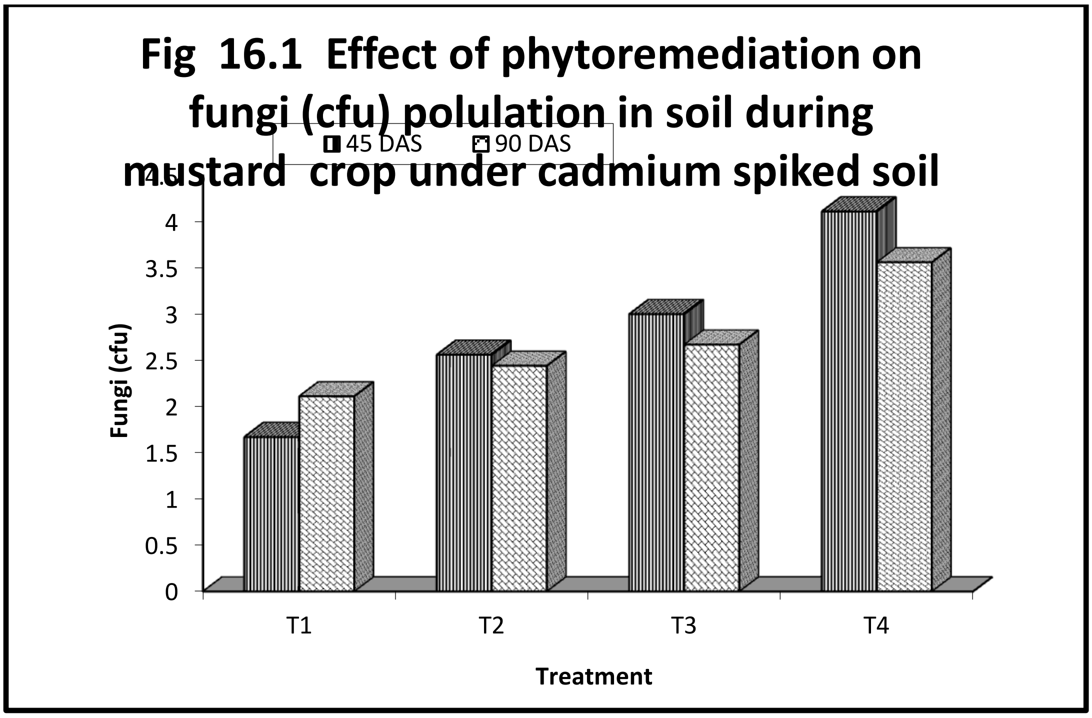


Fig 16.1 Effect of phytoremediation on fungi (cfu) polulation in soil during mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

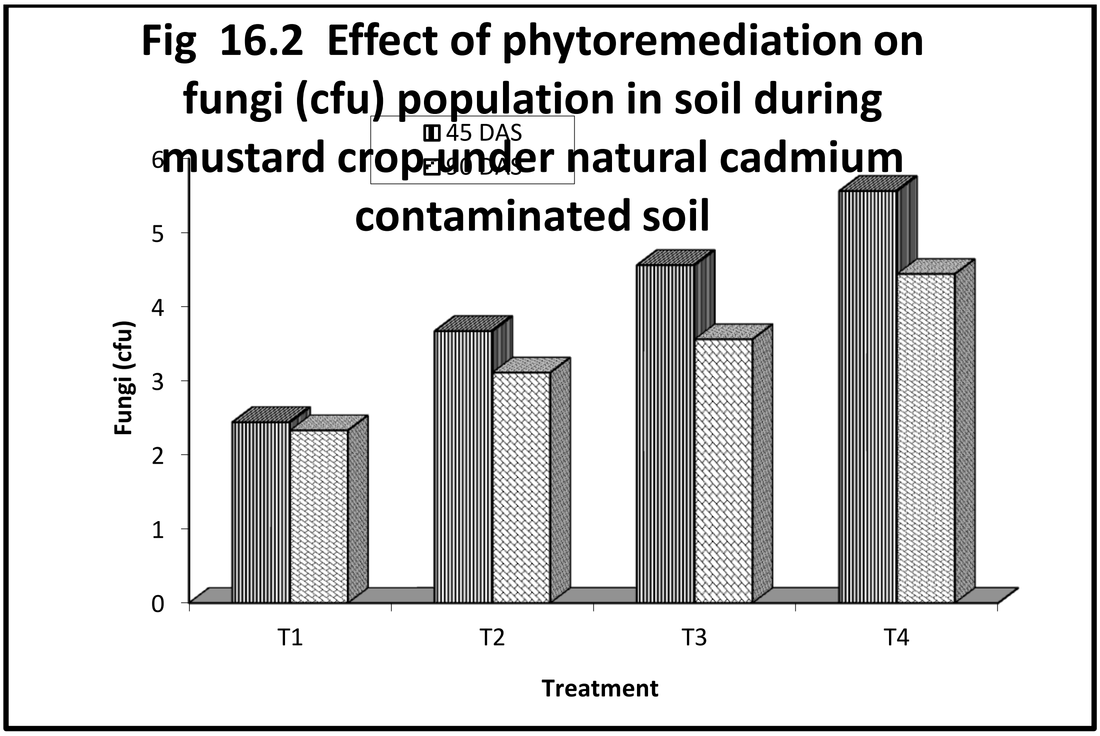


Fig 16.2 Effect of phytoremediation on fungi (cfu) population in soil during mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 17.1 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during mustard crop under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	8.44	11.67	10.06
T2	**RDF+SSP**	10.44	12.33	11.39
T3	**RDF+AM**	12.11	13.22	12.67
T4	**RDF+SSP+AM**	14.44	14.67	14.56
Mean		11.36	12.97	
		SEm±	**CD at 5% level**	
Treatment (T)		0.488	1.463	**SIG**
Stage (S)		0.345	1.034	**SIG**
Interaction (TxS)		0.598	1.791	**NS**

The effects of treatment had significant effect on available bacteria in the mustard which was found in count of 12.67 (cfu) (T3) and 11.39 (cfu) (T2) respectively (Table, Fig 17.1 and Appendix 1.11). The T4 (RDF+AM+SSP) was found most prominent effect of bacteria in count 14.56 (cfu) where as T1 (RDF) was observed in the least bacteria in count of 10.06 (cfu) in the mustard crop. The stages of the crop growth had significant effect of bacteria on the mustard crop. In 90 DAS (12.97 cfu) the mustard had much the higher bacteria than the 45 DAS (11.36 cfu). There was non-significant role which the interaction of growing stage and treatment in the bacteria of in mustard crop.

Table 17.2 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	14.78	12.89	13.83
T2	**RDF+SSP**	15.00	14.11	14.56
T3	**RDF+AM**	16.78	14.22	15.50
T4	**RDF+SSP+AM**	21.44	15.11	18.28
Mean		11.36	12.97	
		SEm±	**CD at 5% level**	
Treatment (T)		0.893	2.676	**SIG**
Stage (S)		0.631	1.892	**SIG**
Interaction (TxS)		1.093	3.278	**NS**

The effects of treatment had significant effect on fungi in the mustard crop which was observed count of 15.50 (cfu) (T3) and 14.56 (cfu) (T2) respectively (Table, Fig

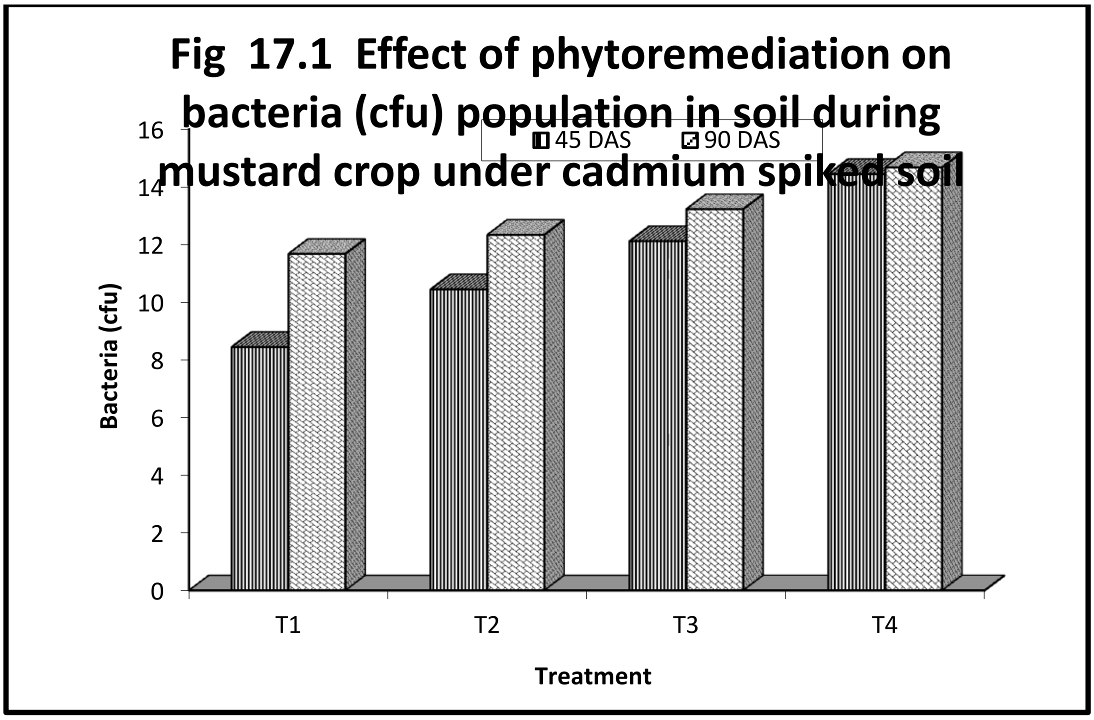


Fig 17.1 Effect of phytoremediation on bacteria (cfu) population in soil during mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

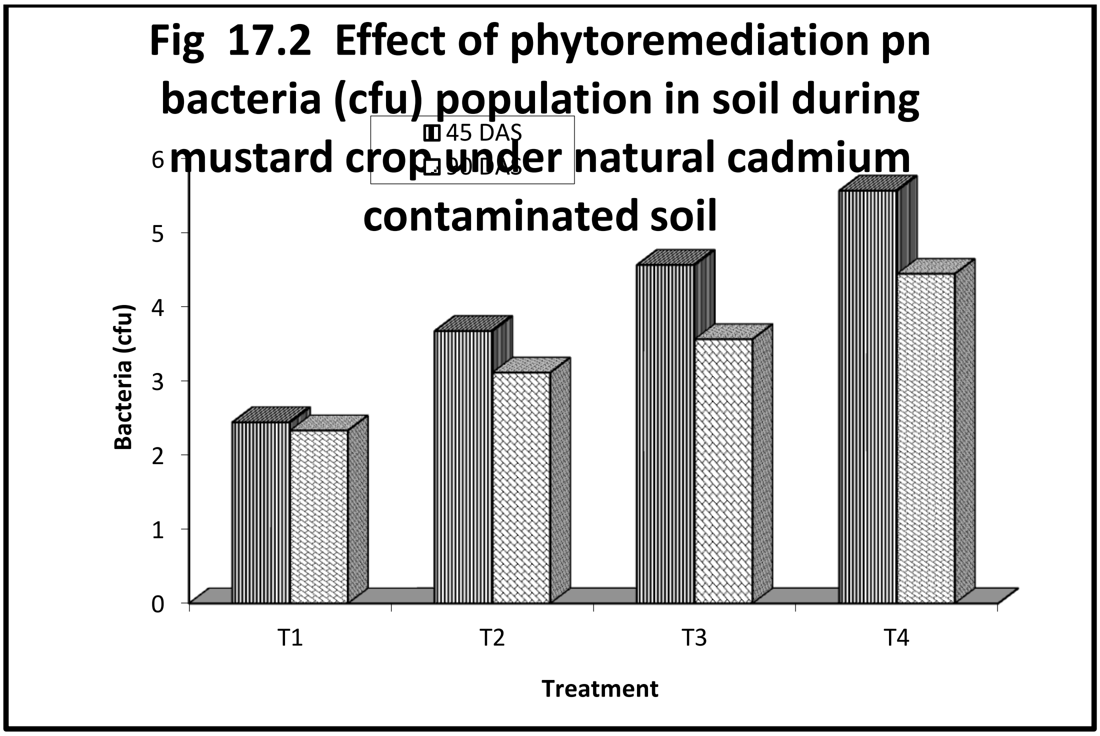


Fig 17.2 Effect of phytoremediation pn bacteria (cfu) population in soil during mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

17.2 and Appendix 1.12). The T4 (RDF+AM+SSP) was found most prominent effect bacteria count of (18.28 cfu) where as T1 (RDF) was found in the least bacteria count (13.83 cfu) in the mustard crop. The stages of the crop growth had significant effect of bacteria on the mustard crop. In 90 DAS (12.97 cfu) the mustard had much the higher bacteria than the 45 DAS (11.36 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

Table 18.1 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during mustard crop under cadmium spiked soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	9.33	11.44	10.39
T2	**RDF+SSP**	12.56	12.56	12.56
T3	**RDF+AM**	14.33	13.44	13.89
T4	**RDF+SSP+AM**	14.89	14.89	14.89
Mean		12.78	13.08	
		SEm±	**CD at 5% level**	
Treatment (T)		0.286	0.858	**SIG**
Stage (S)		0.202	0.607	**NS**
Interaction (TxS)		0.351	1.051	**SIG**

The effects of treatment had significant effect on actinomycetes in the mustard which was found in count of 13.89 (cfu) (T3) and 12.56 (cfu) (T2) respectively (Table, Fig 18.1 and Appendix 1.11) whereasT4 (RDF+AM+SSP) was observed most prominent effect of actinomycetes in count 14.89 (cfu) while T1 (RDF) was found in the least actinomycetes in count of 10.39 (cfu) in the mustard crop. The stages of the crop growth have non-significant effect of actinomycetes on the mustard crop. In this study the interaction between growing stage and treatment due to actinomycetes in AM addition had significant role on mustard crop actinomycetes in cadmium spiked soil. In 90 DAS among treatment the T4 (14.89 cfu) was found superior than other treatment combination with growing stage of mustard the actinomycetes decrease and which was found highest in T4 treatment.

Table 18.2 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	13.89	14.67	14.28
T2	**RDF+SSP**	14.33	17.78	16.06
T3	**RDF+AM**	16.00	18.44	17.22
T4	**RDF+SSP+AM**	18.33	20.11	19.22
Mean		15.64	17.75	
		SEm±	**CD at 5% level**	
Treatment (T)		0.581	1.742	**SIG**
Stage (S)		0.411	1.232	**SIG**
Interaction (TxS)		0.712	2.133	**NS**

The effects of treatment had significant effect on actinomycetes in the mustard crop which was observed count of 17.22 (cfu) (T3) and 16.06 (cfu) (T2) respectively (Table, Fig 18.2 and Appendix 1.12) whereas T4 (RDF+AM+SSP) was found most prominent effect actinomycetes count of (19.22 cfu) while T1 (RDF) was found in the least actinomycetes count (14.28 cfu) in the mustard crop. The stages of the crop growth had significant effect of actinomycetes on the mustard crop. In 90 DAS (17.75 cfu) the mustard had much the higher available actinomycetes than the 45 DAS (15.64 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

Table 19.1 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during mustard crop under cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	18.56	24.56	21.56
T2	**RDF+SSP**	21.11	27.00	24.06
T3	**RDF+AM**	23.78	31.00	27.39
T4	**RDF+SSP+AM**	26.67	33.11	29.89
Mean		22.53	28.92	
		SEm±	**CD at 5% level**	
Treatment (T)		1.603	4.805	**SIG**
Stage (S)		1.133	3.397	**SIG**
Interaction (TxS)		1.963	5.884	**NS**

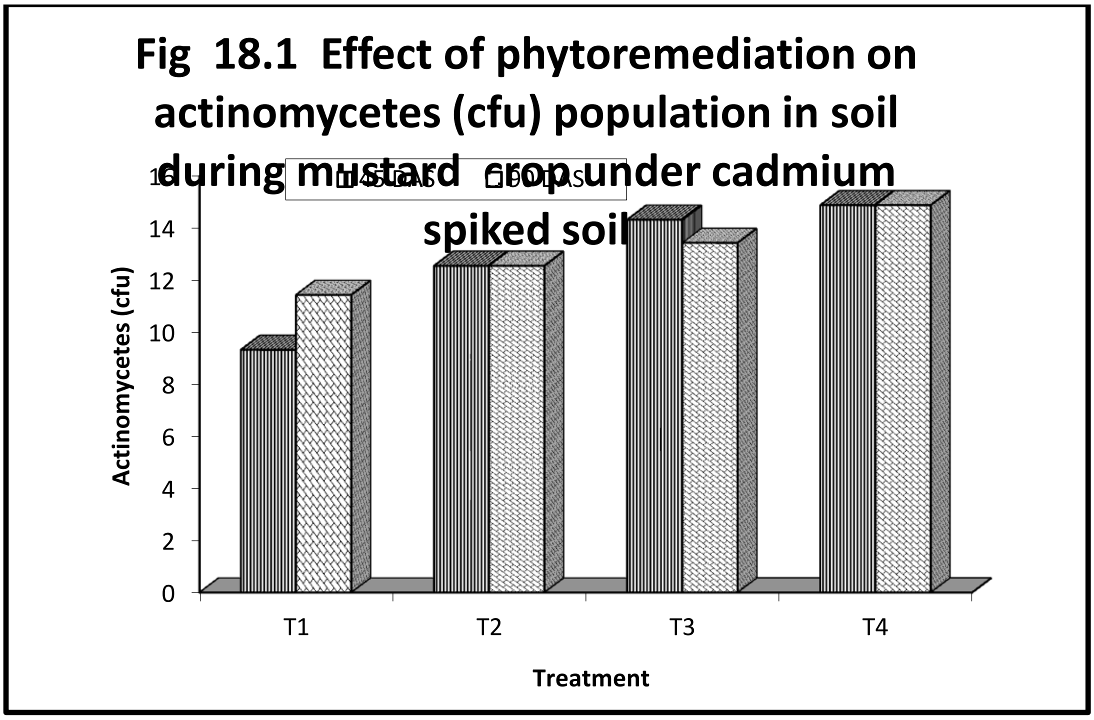


Fig 18.1 Effect of phytoremediation on actinomycetes (cfu) population in soil during mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

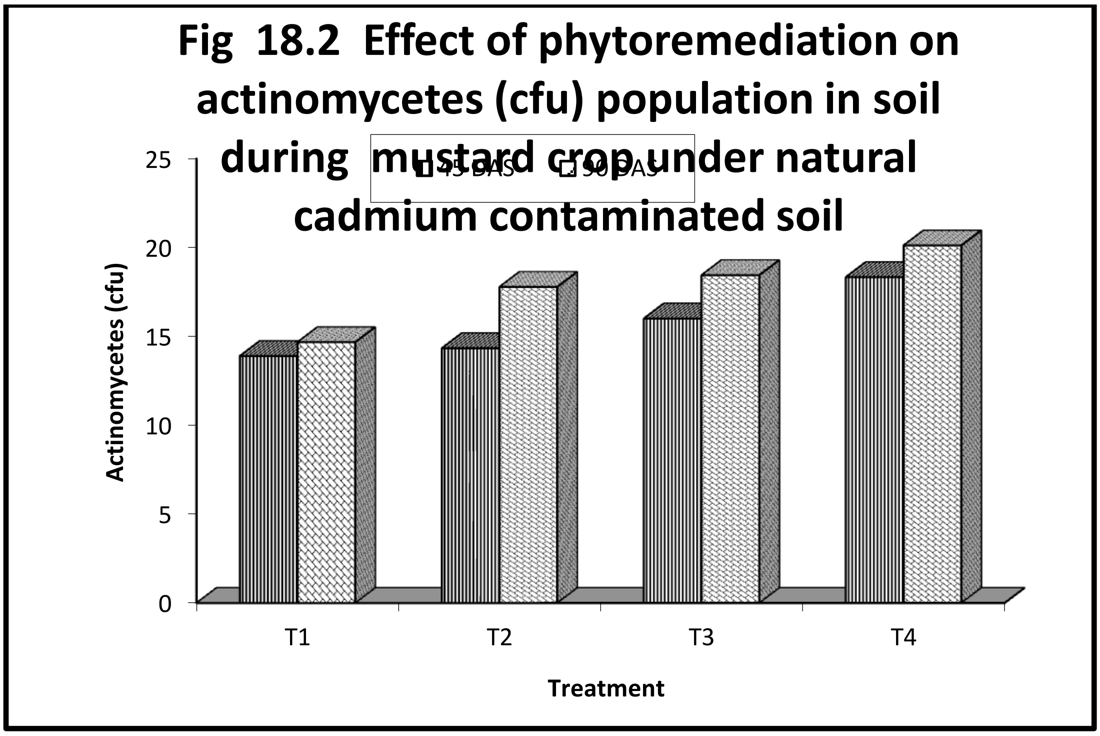


Fig 18.2 Effect of phytoremediation on actinomycetes (cfu) population in soil during mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on N-fixer in the mustard which was found in count of 27.39 (cfu) (T3) and 24.06 (cfu) (T2) respectively (Table, Fig 19.1 and Appendix 1.11). The T4 (RDF+AM+SSP) was found most prominent effect of N-fixer in count 29.89 (cfu) where as T1 (RDF) was observed in the least available N-fixer in count of 21.56 (cfu) in the mustard crop. The stages of the crop growth had significant effect of N-fixer on the mustard crop. In 90 DAS (28.92 cfu) the mustard had much the higher N-fixer than the 45 DAS (22.53 cfu). There was non-significant role which the interaction of growing stage and treatment in the N-fixer of in mustard crop.

Table 19.2 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	20.33	27.89	24.11
T2	**RDF+SSP**	25.44	29.89	27.67
T3	**RDF+AM**	27.00	31.78	29.39
T4	**RDF+SSP+AM**	30.67	34.11	32.39
Mean		25.86	30.92	
		SEm±	**CD at 5% level**	
Treatment (T)		1.320	3.958	**SIG**
Stage (S)		0.933	2.798	**SIG**
Interaction (TxS)		1.617	4.847	**NS**

The effects of treatment had significant effect on N-fixer in the mustard crop which was observed count of 29.39 (cfu) (T3) and 27.67 (cfu) (T2) respectively (Table, Fig 19.2 and Appendix 1.12). The T4 (RDF+AM+SSP) was found most prominent effect N-fixer count of (32.39 cfu) where as T1 (RDF) was found in the least N-fixer count (24.11cfu) in the mustard crop. The stages of the crop growth had significant effect of N-fixer on the mustard crop. In 90 DAS (30.92 cfu) the mustard have much the higher N-fixer than the 45 DAS (25.86 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

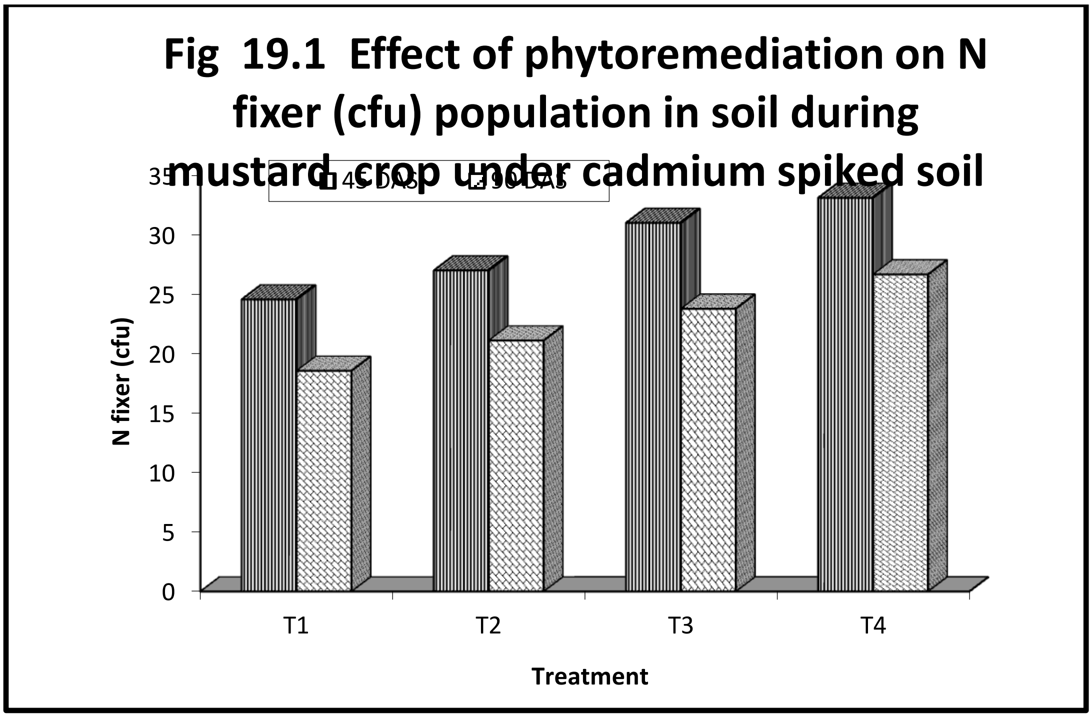


Fig 19.1 Effect of phytoremediation on N fixer (cfu) population in soil during mustard crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

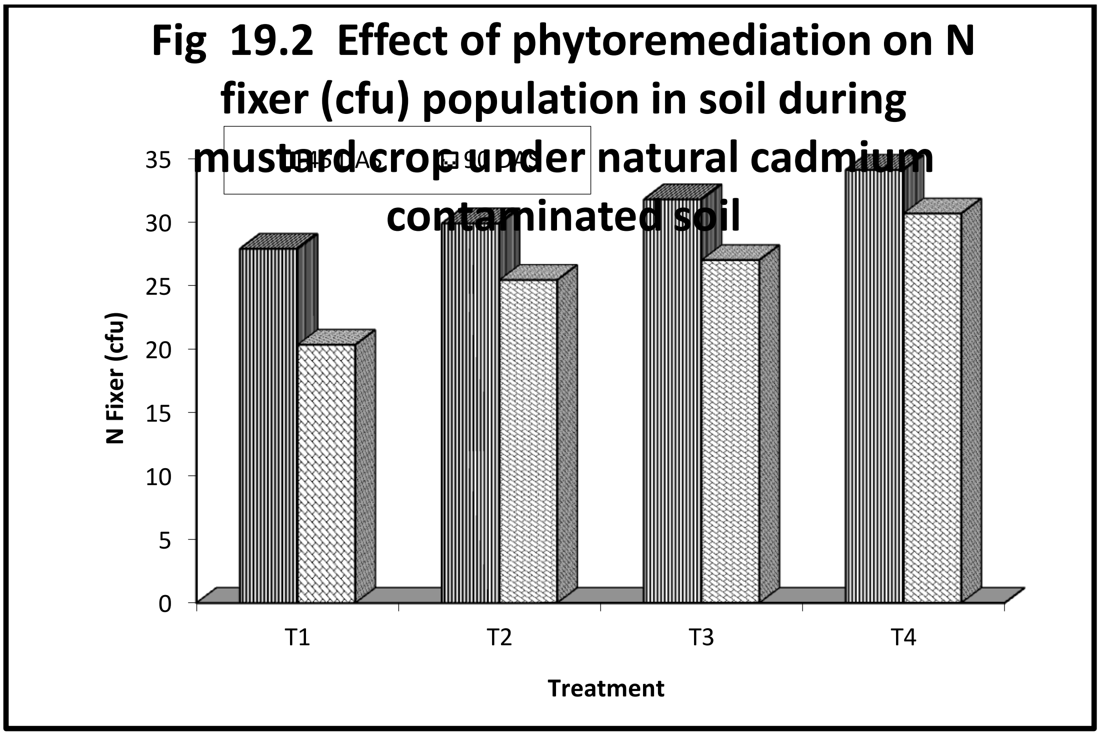


Fig 19.2 Effect of phytoremediation on N fixer (cfu) population in soil during mustard crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 20.1 Effect of phytoremediation on N content (%) in spinach plant crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)			
		45 DAS	60 DAS	90 DAS	Mean
T1	**RDF**	0.74	0.14	0.65	0.40
T2	**RDF+SSP**	0.86	0.15	0.74	0.45
T3	**RDF+AM**	0.79	0.16	0.86	0.51
T4	**RDF+SSP+AM**	0.97	0.21	0.99	0.60
Mean		0.84	0.17	0.81	
		SEm±		**CD at 5% level**	
Treatment (T)		0.006		0.019	**NS**
Stage (S)		0.006		0.017	**SIG**
Interaction (TxS)		0.011		0.033	**SIG**

The effects of treatment had non-significant effect on N content in the spinach plant (Table, Fig 20.1 and Appendix 1.13). The stages of the crop growth had significant effect of N accumulation on the spinach plant. In 45 DAS (0.84%) the plant had much the higher N accumulation than the 60 DAS (0.17%) and 90 DAS (0.81%). In this study the interaction between growing stage and treatment due to N accumulation AM addition was found significant role on spinach plant N accumulation in cadmium spiked soil. In 90 DAS among treatment the T4 (0.99%) was found superior than other treatment combination with growing stage of spinach plant the N accumulation decrease and which was observed highest in T4 treatment.

Table 20.2 Effect of phytoremediation on N content (%) in spinach plant crop under naturally contaminated soil

Treatment		Stage of plant growth (spinach)			
		45 DAS	60 DAS	90 DAS	Mean
T1	**RDF**	0.89	0.14	0.91	0.52
T2	**RDF+SSP**	0.94	0.16	1.09	0.63
T3	**RDF+AM**	0.97	0.21	1.17	0.69
T4	**RDF+SSP+AM**	1.13	0.24	1.36	0.80
Mean		0.98	0.19	1.13	
		SEm±		**CD at 5% level**	
Treatment (T)		0.009		0.028	NS
Stage (S)		0.008		0.024	SIG
Interaction (TxS)		0.016		0.048	SIG

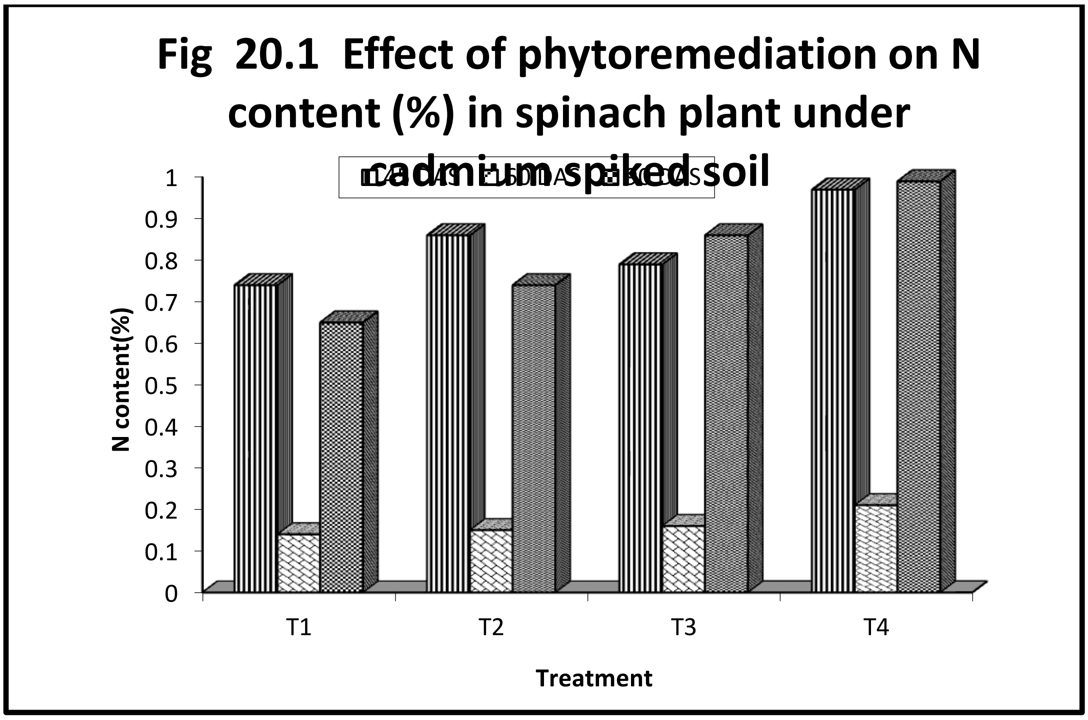


Fig 20.1 Effect of phytoremediation on N content (%) in spinach plant under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

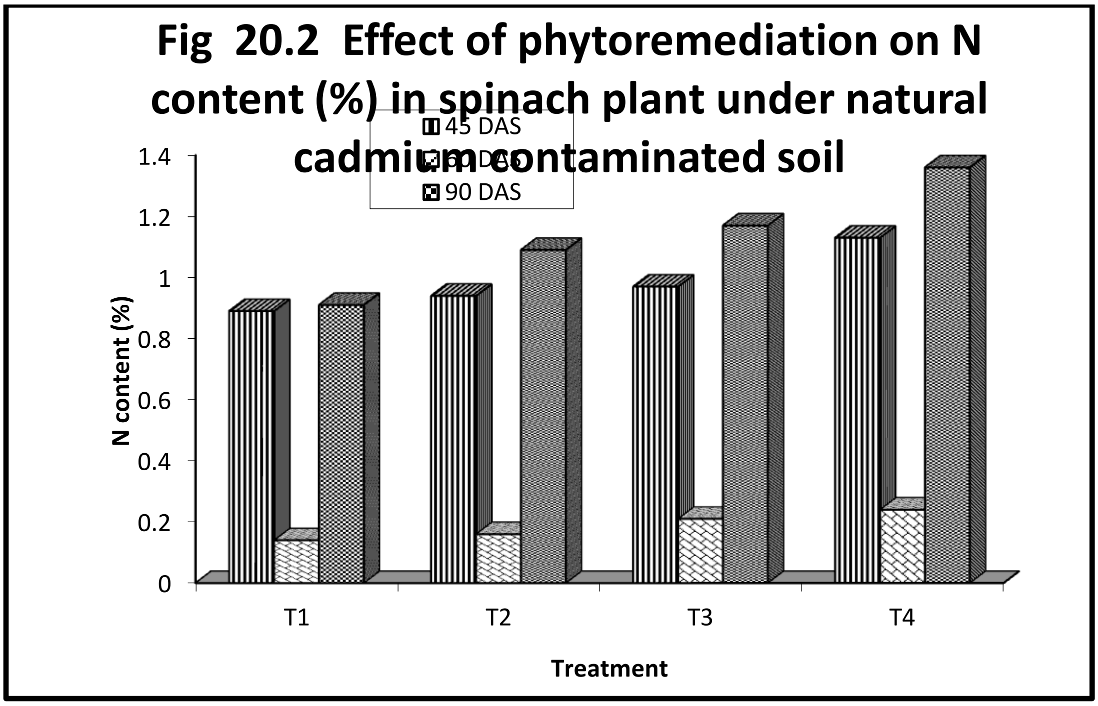


Fig 20.2 Effect of phytoremediation on N content (%) in spinach plant under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on N accumulation in the spinach plant (Table, Fig 20.2 and Appendix 1.14). The stages of the crop growth had significant effect of N accumulation on the plant. In 90 DAS (1.13%) the plant had much the higher N accumulation than the 45 DAS (0.98%) and 60 DAS (0.19%). In this study the interaction between growing stage and treatment due to AM addition had significant role on spinach plant N accumulation in naturally cadmium contaminated. In 90 DAS among treatment the T4 (1.36%) was found superior than other treatment combination with growing stage of spinach plant the N accumulation decrease and which was found highest in T4 treatment.

Table 20.3 Effect of phytoremediation on N content (%) in spinach root under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.028	0.075	0.051
T2	**RDF+SSP**	0.065	0.121	0.093
T3	**RDF+AM**	0.079	0.106	0.093
T4	**RDF+SSP+AM**	0.131	0.146	0.138
Mean		0.08	0.11	
		SEm±	**CD at 5% level**	
Treatment (T)		0.010	0.030	**SIG**
Stage (S)		0.007	0.021	**SIG**
Interaction (TxS)		0.012	0.036	**NS**

The effects of treatment had significant effect on N accumulation in the spinach root which was found 0.093% (T3) and 0.093% (T2) respectively (Table, Fig 20.3 and Appendix 1.15). The T4 (RDF+AM+SSP) was found most promoted effect N accumulation in the spinach root (0.13%) whereas T1 (RDF) was observed in the least content (0.050%) in the plant. The stages of the crop growth have significant effect of N accumulation on the spinach root. In 90 DAS (0.11%) the plant have much the higher N accumulation than the 45 DAS (0.08%). There was a non significant interaction between the growing stage crop and treatments on N accumulation of in spinach root. Application of mycorrhiza and phosphatic fertilizer significantly influence the N content in the spinach root.

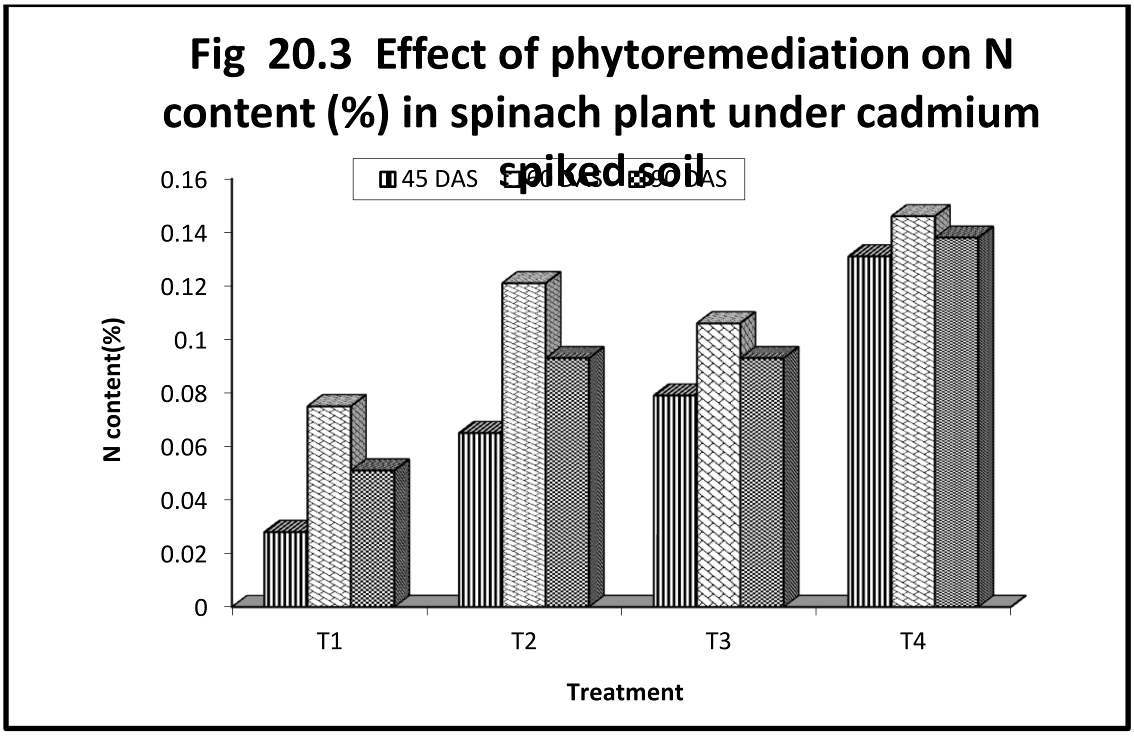


Fig 20.3 Effect of phytoremediation on N content (%) in spinach plant under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

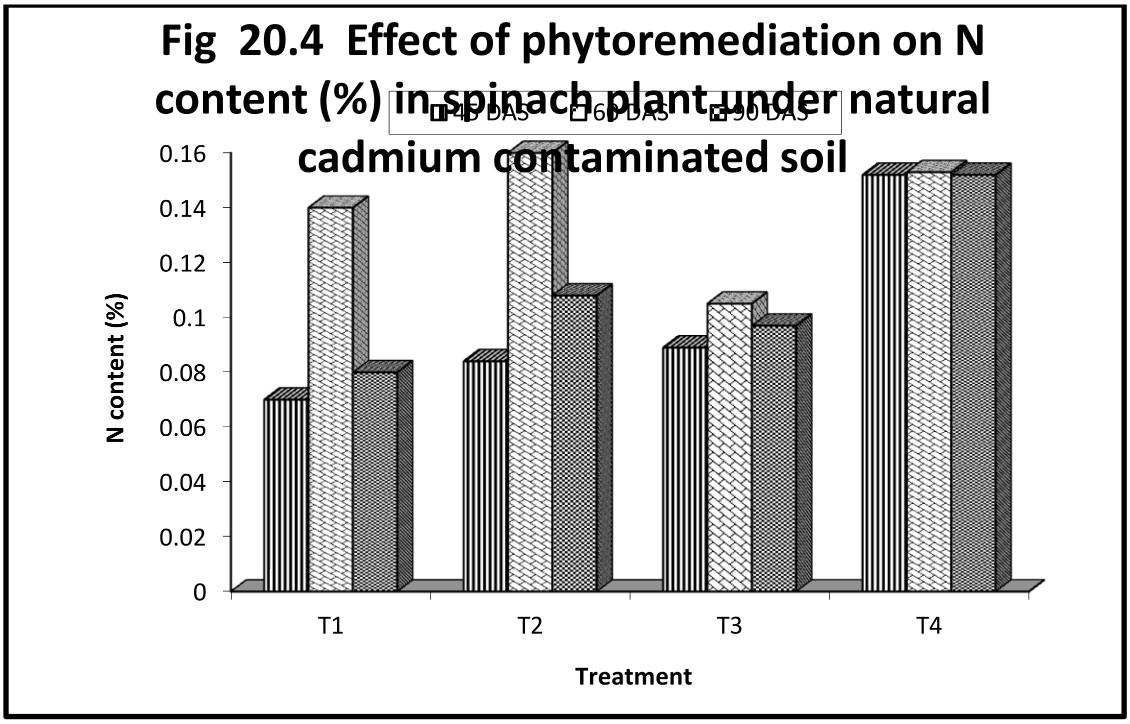


Fig 20.4 Effect of phytoremediation on N content (%) in spinach plant under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 20.4 Effect of phytoremediation on N content (%) in spinach root under naturally cadmium contaminated soil

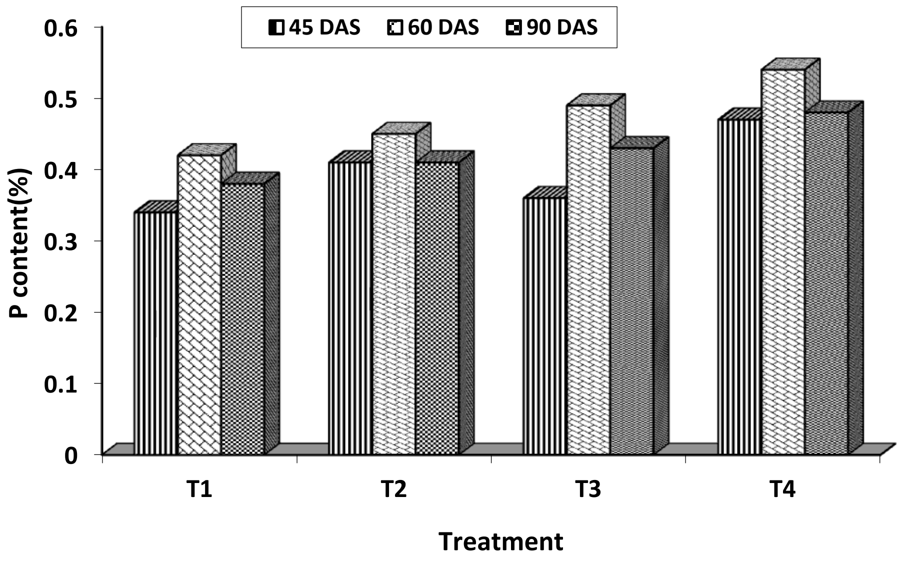
Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.070	0.090	0.080
T2	**RDF+SSP**	0.084	0.133	0.108
T3	**RDF+AM**	0.089	0.105	0.097
T4	**RDF+SSP+AM**	0.152	0.153	0.152
Mean		0.10	0.12	
		SEm±	**CD at 5% level**	
Treatment (T)		0.007	0.021	**SIG**
Stage (S)		0.005	0.015	**SIG**
Interaction (TxS)		0.008	0.025	**NS**

The effects of treatment had significant effect on N accumulation in the spinach root which was found 0.097% (T3) and 0.108% (T2) respectively (Table, Fig 20.4 and Appendix 1.16). The T4 (RDF+AM+SSP) was found most promoted effect N accumulation in the plant (0.152%) where as T1 (RDF) was observed in the least content (0.080%) in the plant. The stages of the crop growth had significant effect of N accumulation on the plant. In 90 DAS (0.12%) the plant had much the higher N accumulation than the 45 DAS (0.10%). The interaction effect between growing stage and treatment was observed in non-significant.

Table 21.1 Effect of phytoremediation on P content (%) in spinach plant under cadmium spiked soil

Treatment		Stage of plant growth (spinach)			
		45 DAS	**60 DAS**	**90 DAS**	**Mean**
T1	**RDF**	0.34	0.42	0.38	0.40
T2	**RDF+SSP**	0.41	0.45	0.41	0.43
T3	**RDF+AM**	0.36	0.49	0.43	0.46
T4	**RDF+SSP+AM**	0.47	0.54	0.48	0.51
Mean		0.40	0.48	0.43	
		SEm±		**CD at 5% level**	
Treatment (T)		0.007		0.019	**NS**
Stage (S)		0.006		0.017	**SIG**
Interaction (TxS)		0.011		0.034	**SIG**

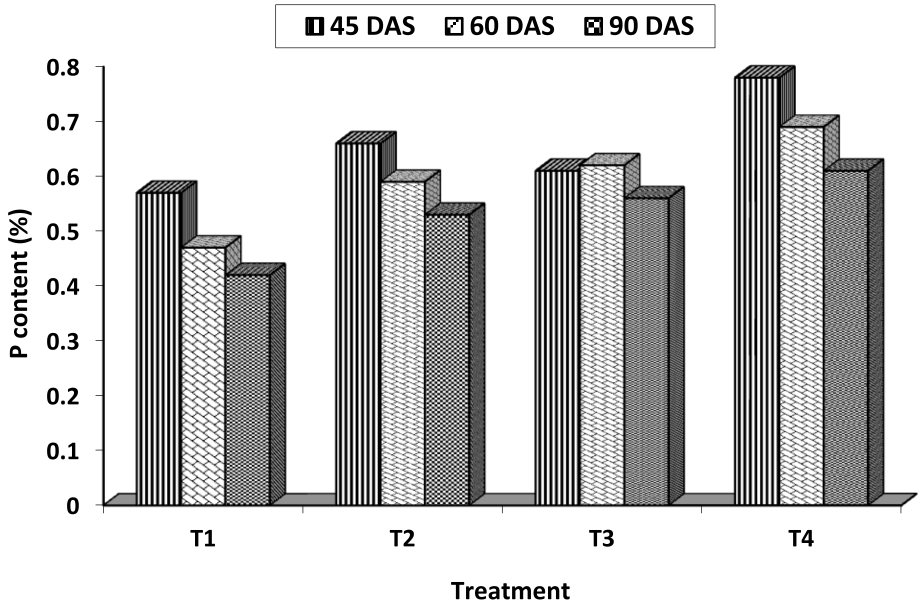
Fig 21.1 Effect of phytoremediation on P content (%) in spinach plant under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 21.2 Effect of phytoremediation on P content (%) in spinach plant under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on P content in the spinach plant (Table, Fig 21.1 and Appendix 1.13). The stages of the crop growth had significant effect of P accumulation on the spinach plant. In 60 DAS (0.48%) the plant had much the higher P content than the 90 DAS (0.43%) and 45 DAS (0.40%). In this study the interaction between growing stage and treatment due to P content AM+SSP addition had significant role on spinach plant P content in cadmium spiked soil. In 60 DAS among treatment the T4 (0.54%) was found superior than other treatment combination with growing stage of spinach plant the P content decrease and which was observed highest in T4 treatment.

Table 21.2 Effect of phytoremediation on P content (%) in spinach plant under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**			
		45 DAS	**60 DAS**	**90 DAS**	**Mean**
T1	**RDF**	0.57	0.47	0.42	0.44
T2	**RDF+SSP**	0.66	0.59	0.53	0.56
T3	**RDF+AM**	0.61	0.62	0.56	0.59
T4	**RDF+SSP+AM**	0.78	0.69	0.61	0.65
Mean		0.66	0.59	0.53	
		SEm±		**CD at 5% level**	
Treatment (T)		0.011		0.033	**NS**
Stage (S)		0.010		0.029	**SIG**
Interaction (TxS)		0.019		0.057	**SIG**

The effects of treatment had non-significant effect on P content in the spinach plant (Table, Fig 21.2 and Appendix 1.14). The stages of the crop growth had significant effect of P content on the plant. In 45 DAS (0.66%) the plant had much the higher P content than the 60 DAS (0.59%) and 90 DAS (0.53%). In this study the interaction between growing stage and treatment due to P content AM+SSP addition had significant role on spinach plant P content in naturally cadmium contaminated. In 45 DAS among treatment the T4 (0.78%) was found superior than other treatment combination with growing stage of spinach plant the P content decrease and which was found highest in T4 treatment.

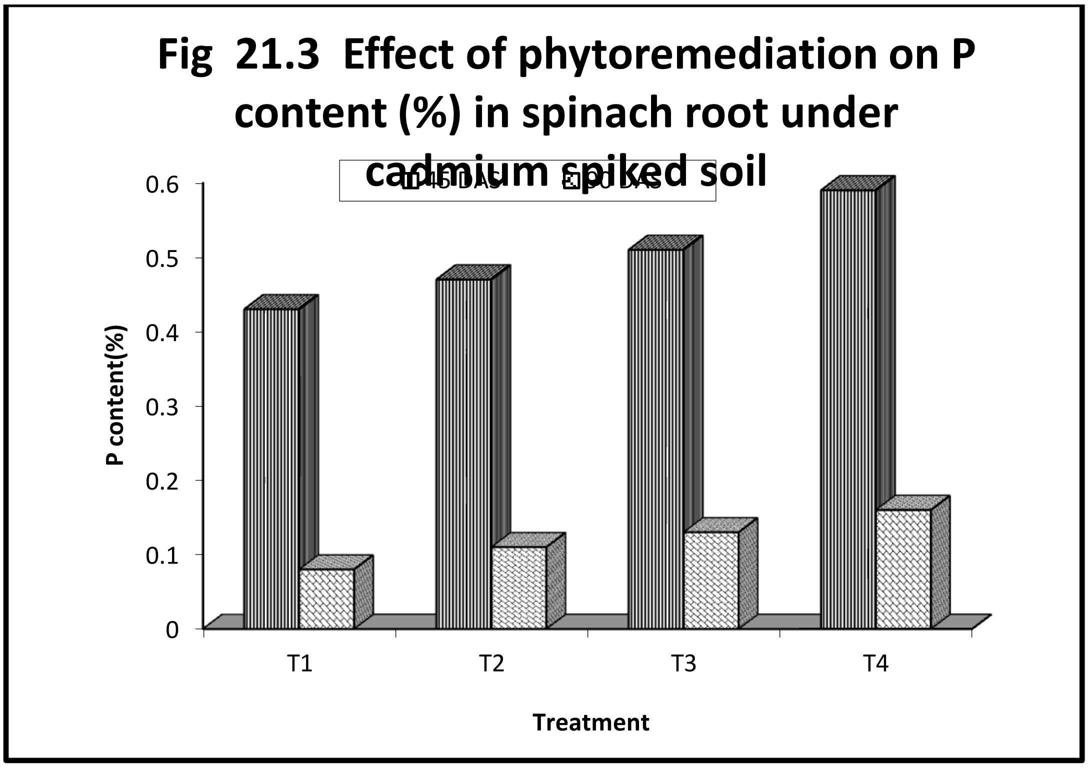


Fig 21.3 Effect of phytoremediation on P content (%) in spinach root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

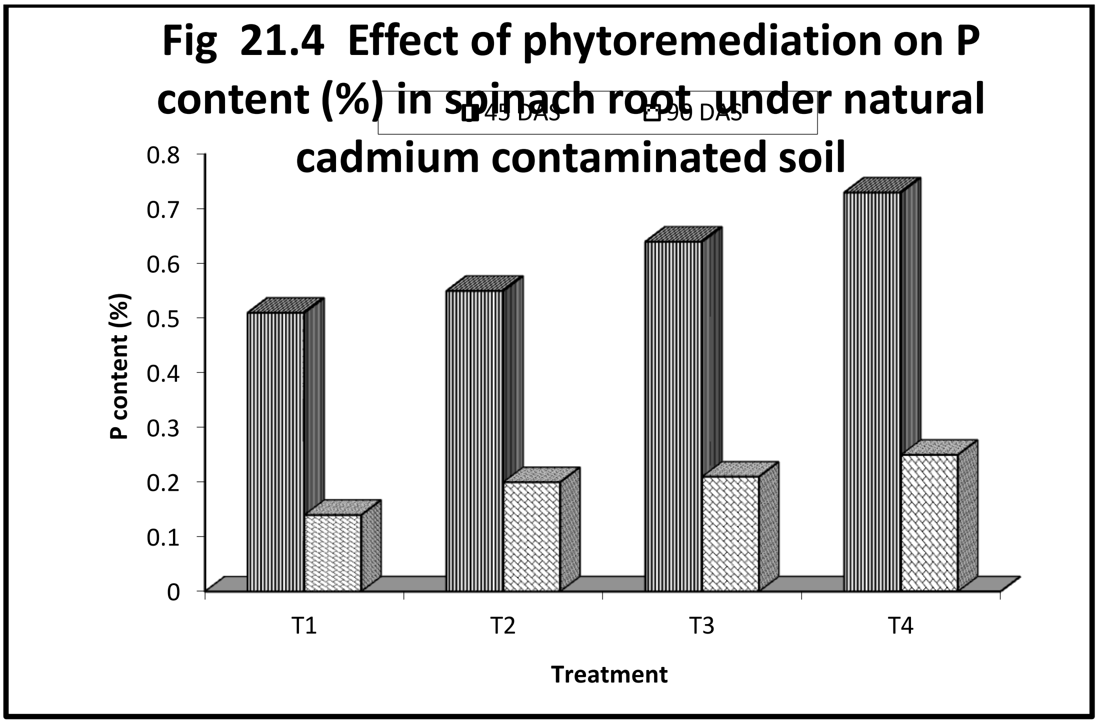


Fig 21.4 Effect of phytoremediation on P content (%) in spinach root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 21.3 Effect of phytoremediation on P content (%) in spinach root under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.43	0.08	0.26
T2	**RDF+SSP**	0.47	0.11	0.29
T3	**RDF+AM**	0.51	0.13	0.32
T4	**RDF+SSP+AM**	0.59	0.16	0.37
Mean		0.50	0.12	
		SEm±	**CD at 5% level**	
Treatment (T)		0.095	0.285	**NS**
Stage (S)		0.067	0.201	**SIG**
Interaction (TxS)		0.116	0.349	**NS**

The effects of treatment had non-significant effect on P content in the spinach plant root (Table, Fig 21.3 and Appendix 1.15). The stages of the crop growth had significant effect on P content on the plant root. In 90 DAS (0.12%) the plant root had much the lower P content than the 45 DAS (0.50%). There was no significant role which the interaction of growing stage and treatment in the P content of in spinach root part.

Table 21.4 Effect of phytoremediation on P content (%) in spinach root under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.51	0.14	0.33
T2	**RDF+SSP**	0.55	0.20	0.38
T3	**RDF+AM**	0.64	0.21	0.43
T4	**RDF+SSP+AM**	0.73	0.25	0.49
Mean		0.61	0.20	
		SEm±	**CD at 5% level**	
Treatment (T)		0.103	0.307	**NS**
Stage (S)		0.073	0.217	**SIG**
Interaction (TxS)		0.126	0.377	**NS**

The effects of treatment had non-significant effect on P content in the spinach plant root (Table, Fig 21.4 and Appendix 1.16). The stages of the crop growth had significant effect on P content on the plant root. In 90 DAS (0.20%) the plant root had much the lower P content than the 45 DAS (0.61%). The interaction effect between growing stage and treatment was observed in non-significant.

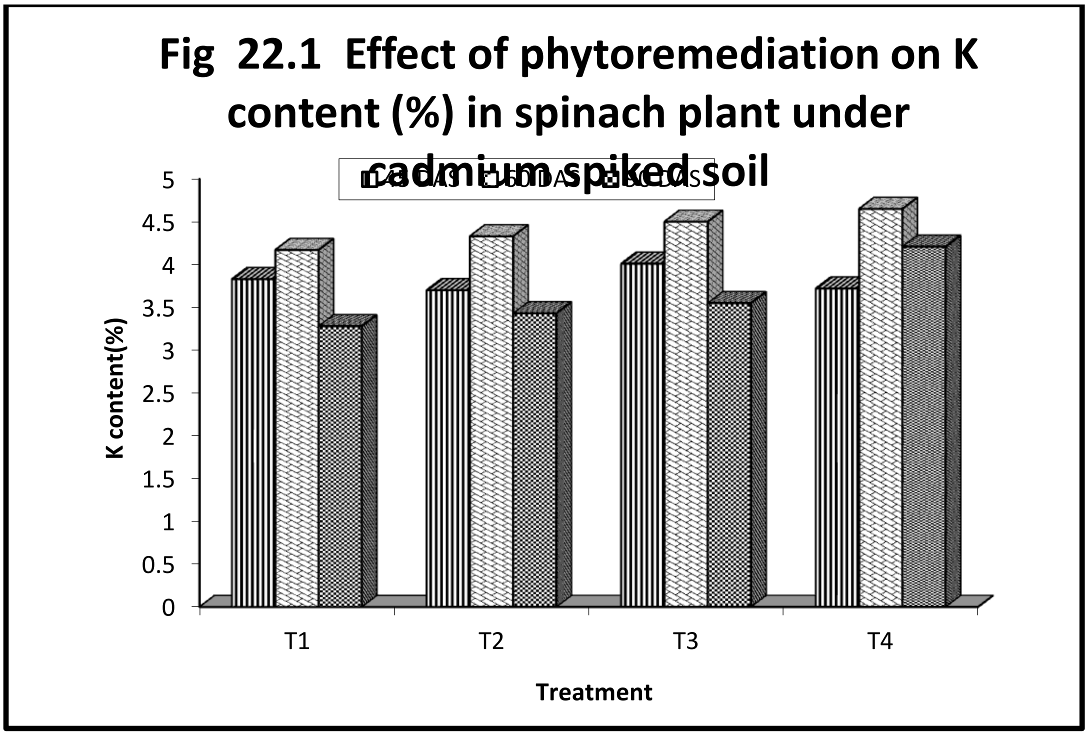
Table 22.1 Effect of phytoremediation on K content (%) in spinach plant under cadmium spiked soil

Treatment		Stage of plant growth (spinach)			
		45 DAS	60 DAS	90 DAS	Mean
T1	RDF	3.83	4.17	3.28	3.72
T2	RDF+SSP	3.70	4.33	3.43	3.88
T3	RDF+AM	4.01	4.50	3.55	4.02
T4	RDF+SSP+AM	3.72	4.65	4.21	4.43
Mean		3.82	4.41	3.62	
		SEm±	CD at 5% level		
Treatment (T)		0.035	0.105	NS	
Stage (S)		0.030	0.091	SIG	
Interaction (TxS)		0.061	0.182	SIG	

The effects of treatment had non-significant effect on K content in the spinach plant (Table, Fig 22.1 and Appendix 1.13). The stages of the crop growth had significant effect of K accumulation on the spinach plant. In 60 DAS (4.41%) the plant had much the higher K content than the 45 DAS (3.82%) and 90 DAS (3.62%). In this study the interaction between growing stage and treatment due to K content AM+SSP addition had significant role on spinach plant K content in cadmium spiked soil. In 60 DAS among treatment the T4 (4.65%) was found superior than other treatment combination with growing stage of spinach plant the K content decrease and which was found highest in T4 treatment.

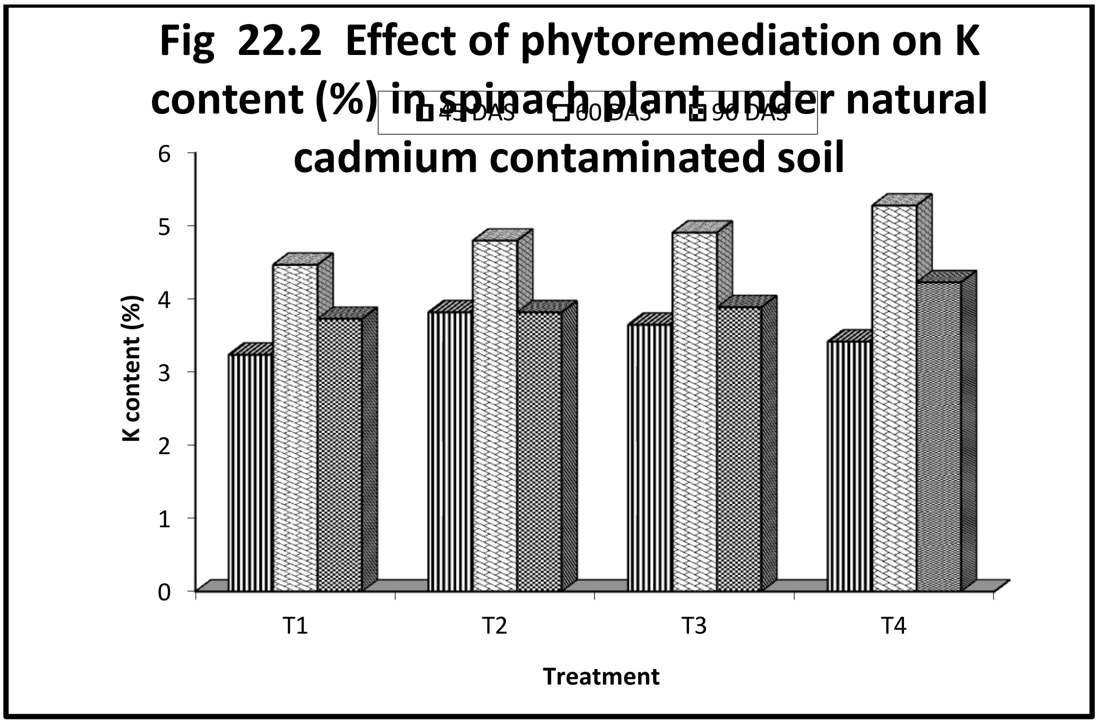
Table 22.2 Effect of phytoremediation on K content (%) in spinach plant under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)			
		45 DAS	60 DAS	90 DAS	Mean
T1	RDF	3.24	4.47	3.73	4.10
T2	RDF+SSP	3.82	4.80	3.82	4.31
T3	RDF+AM	3.65	4.91	3.89	4.40
T4	RDF+SSP+AM	3.42	5.28	4.23	4.75
Mean		3.53	4.87	3.92	
		SEm±	CD at 5% level		
Treatment (T)		0.019	0.058	NS	
Stage (S)		0.017	0.050	SIG	
Interaction (TxS)		0.034	0.101	SIG	



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on K content in the spinach plant (Table, Fig 22.2 and Appendix 1.14). The stages of the crop growth had significant effect of K content on the plant. In 60 DAS (4.87%) the plant was much the higher K content than the 90 DAS (3.92%) and 45 DAS (3.53%). In this study the interaction between growing stage and treatment due to K content AM+SSP addition had significant role on spinach plant K content in naturally cadmium contaminated. In 60 DAS among treatment the T4 (5.28%) was found superior than other treatment combination with growing stage of spinach plant the K content decrease and which was found highest in T4 treatment.

Table 22.3 Effect of phytoremediation on K content (%) in spinach root under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	2.13	0.69	1.41
T2	**RDF+SSP**	2.24	0.79	1.52
T3	**RDF+AM**	2.33	0.87	1.60
T4	**RDF+SSP+AM**	2.48	0.94	1.71
Mean		2.30	0.82	
		SEm±	**CD at 5% level**	
Treatment (T)		0.368	1.103	**NS**
Stage (S)		0.260	0.780	**SIG**
Interaction (TxS)		0.450	1.350	**NS**

The effects of treatment had non-significant effect on K content in the spinach plant root (Table, Fig 22.3 and Appendix 1.15). The stages of the crop growth had significant effect on K content on the plant root. In 90 DAS (0.82%) the plant root was much the lower K content than the 45 DAS (2.30%). There was no significant role which the interaction of growing stage and treatment in the K content of in spinach root part.

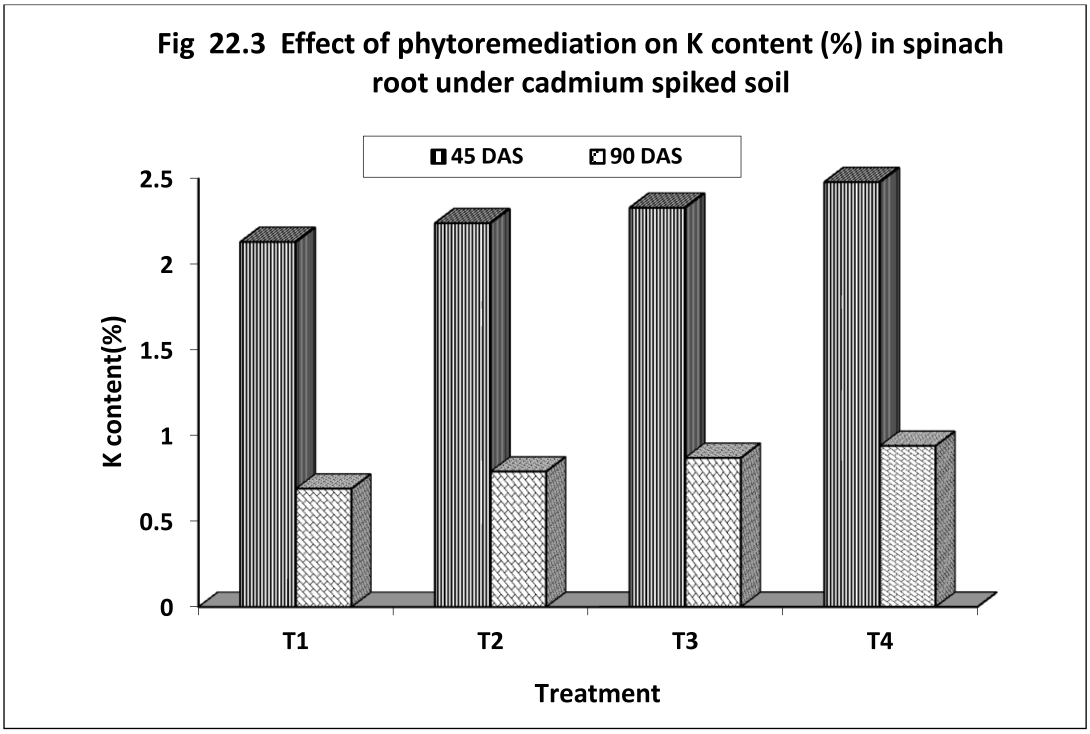


Fig 22.3 Effect of phytoremediation on K content (%) in spinach root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

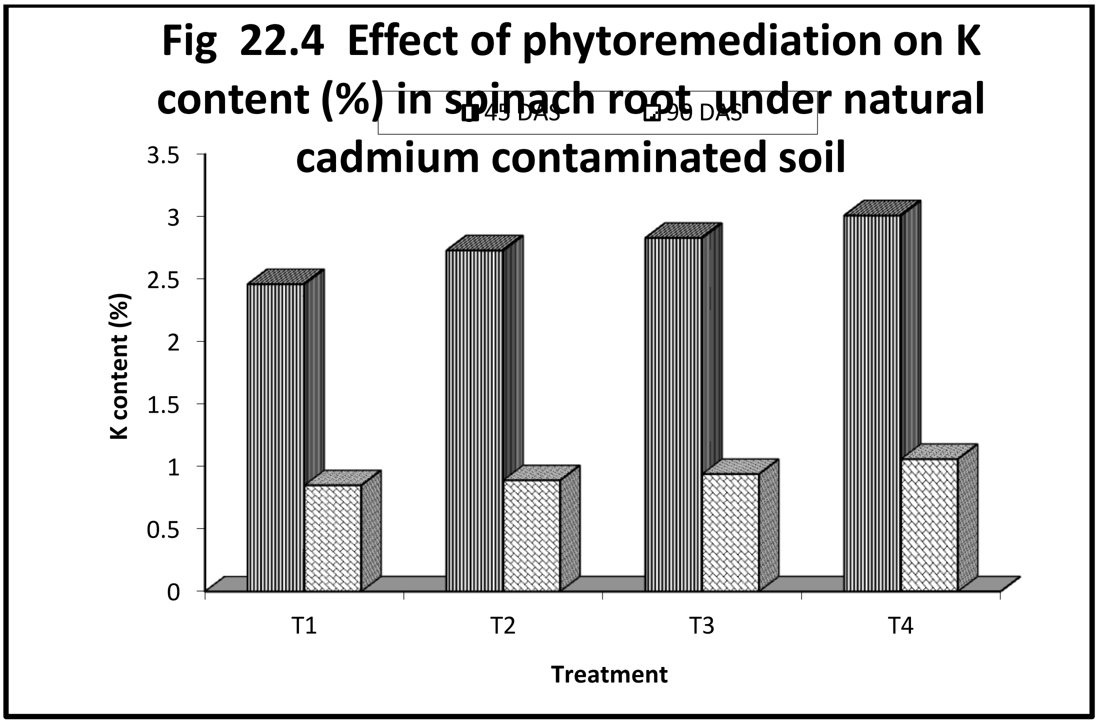


Fig 22.4 Effect of phytoremediation on K content (%) in spinach root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 22.4 Effect of phytoremediation on K content (%) in spinach root under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	90 DAS	Mean
T1	**RDF**	2.46	0.85	1.66
T2	**RDF+SSP**	2.73	0.89	1.81
T3	**RDF+AM**	2.83	0.94	1.88
T4	**RDF+SSP+AM**	3.01	1.06	2.04
Mean		2.76	0.94	
		SEm±	**CD at 5% level**	
Treatment (T)		0.457	1.371	**NS**
Stage (S)		0.323	0.969	**SIG**
Interaction (TxS)		0.560	1.679	**NS**

The effects of treatment had non-significant effect on K content in the spinach plant root (Table, Fig 22.4 and Appendix 1.16). The stages of the crop growth had significant effect on K content on the plant root. In 90 DAS (0.94%) the plant root had much the lower K content than the 45 DAS (2.76%). The interaction effect between growing stage and treatment was observed in non-significant.

Table 23.1 Effect of phytoremediation on S content (%) in spinach plant under cadmium spiked soil

Treatment		Stage of plant growth (spinach)			
		45 DAS	60 DAS	90 DAS	Mean
T1	**RDF**	0.35	0.37	0.28	0.33
T2	**RDF+SSP**	0.38	0.45	0.41	0.43
T3	**RDF+AM**	0.39	0.56	0.33	0.44
T4	**RDF+SSP+AM**	0.40	0.64	0.27	0.45
Mean		0.38	0.51	0.32	
		SEm±	**CD at 5% level**		
Treatment (T)		0.016	0.048	**NS**	
Stage (S)		0.014	0.042	**SIG**	
Interaction (TxS)		0.028	0.083	**SIG**	

The effects of treatment had non-significant effect on S content in the spinach plant (Table, Fig 23.1 and Appendix 1.13). The stages of the crop growth had significant effect of S accumulation on the spinach plant. In 60 DAS (0.51%) the plant was much the higher S content than the 45 DAS (0.38%) and 90 DAS (0.32%).

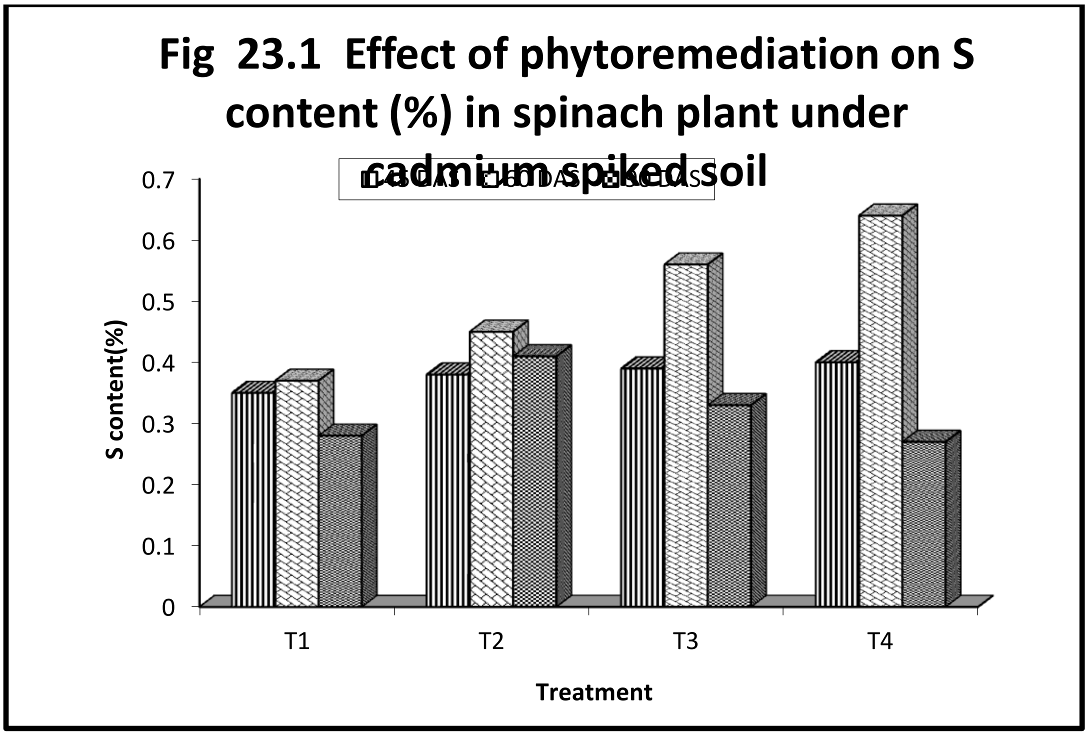


Fig 23.1 Effect of phytoremediation on S content (%) in spinach plant under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

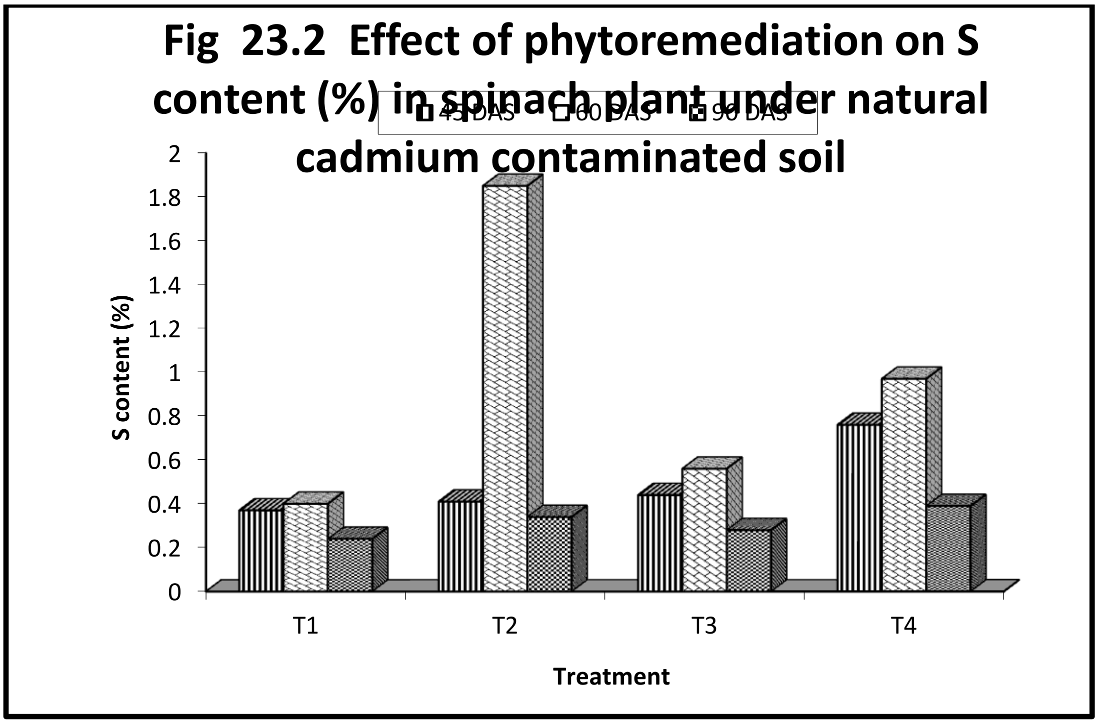


Fig 23.2 Effect of phytoremediation on S content (%) in spinach plant under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

In this study the interaction between growing stage and treatment due to S content AM+SSP addition have significant role on spinach plant S content in cadmium spiked soil. In 60 DAS among treatment the T4 (0.64%) was found superior than other treatment combination with growing stage of spinach plant the S content decrease and which was found highest in T4 treatment.

Table 23.2 Effect of phytoremediation on S content (%) in spinach plant under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)			
		45 DAS	60 DAS	90 DAS	Mean
T1	**RDF**	0.37	0.40	0.24	0.32
T2	**RDF+SSP**	0.41	1.85	0.34	1.09
T3	**RDF+AM**	0.44	0.56	0.28	0.42
T4	**RDF+SSP+AM**	0.76	0.97	0.39	0.68
Mean		0.50	0.95	0.31	
		SEm±	**CD at 5% level**		
Treatment (T)		0.225	0.674	**NS**	
Stage (S)		0.195	0.583	**NS**	
Interaction (TxS)		0.389	1.167	**SIG**	

The effects of treatment had non-significant effect on S content in the spinach plant (Table, Fig 23.2 and Appendix 1.14). The stages of the crop growth had non-significant effect of S content on the plant. In 60 DAS (0.97%) the plant had much the higher S content than the 45 DAS (0.50%) and 90 DAS (0.31%). In this study the interaction between growing stage and treatment due to S content RDF+SSP addition was found significant role on spinach plant S content in naturally cadmium contaminated soil. In 60 DAS among treatment the T2 (1.85%) was showed superior than other treatment combination with growing stage of spinach plant the S content decrease and which was found highest in T2 treatment.

Table 23.3 Effect of phytoremediation on S content (%) in spinach root under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.07	0.22	0.15
T2	**RDF+SSP**	0.09	0.24	0.17
T3	**RDF+AM**	0.10	0.27	0.19
T4	**RDF+SSP+AM**	0.12	0.35	0.24
Mean		0.10	0.27	
		SEm±	**CD at 5% level**	
Treatment (T)		0.045	0.135	**NS**
Stage (S)		0.032	0.096	**SIG**
Interaction (TxS)		0.055	0.166	**NS**

The effects of treatment had non-significant effect on S content in the spinach plant root (Table, Fig 23.3 and Appendix 1.15). The stages of the crop growth had significant effect on S content on the plant root. In 90 DAS (0.27%) plant root was much the higher S content than the 45 DAS (0.10%). There was no significant role which the interaction of growing stage and treatment in the S content of in spinach root part.

Table 23.4 Effect of phytoremediation on S content (%) in spinach root under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.06	0.26	0.16
T2	**RDF+SSP**	0.10	0.24	0.17
T3	**RDF+AM**	0.11	0.27	0.19
T4	**RDF+SSP+AM**	0.20	0.30	0.25
Mean		0.12	0.27	
		SEm±	**CD at 5% level**	
Treatment (T)		0.038	0.114	**NS**
Stage (S)		0.027	0.081	**SIG**
Interaction (TxS)		0.047	0.140	**NS**

The effects of treatment had non-significant effect on S content in the spinach plant root (Table, Fig 23.4 and Appendix 1.16). The stages of the crop growth had significant effect on S content on the plant root. In 90 DAS (0.27%) plant root was much the higher S content than the 45 DAS (0.20%). The interaction effect between growing stage and treatment was observed in non-significant.

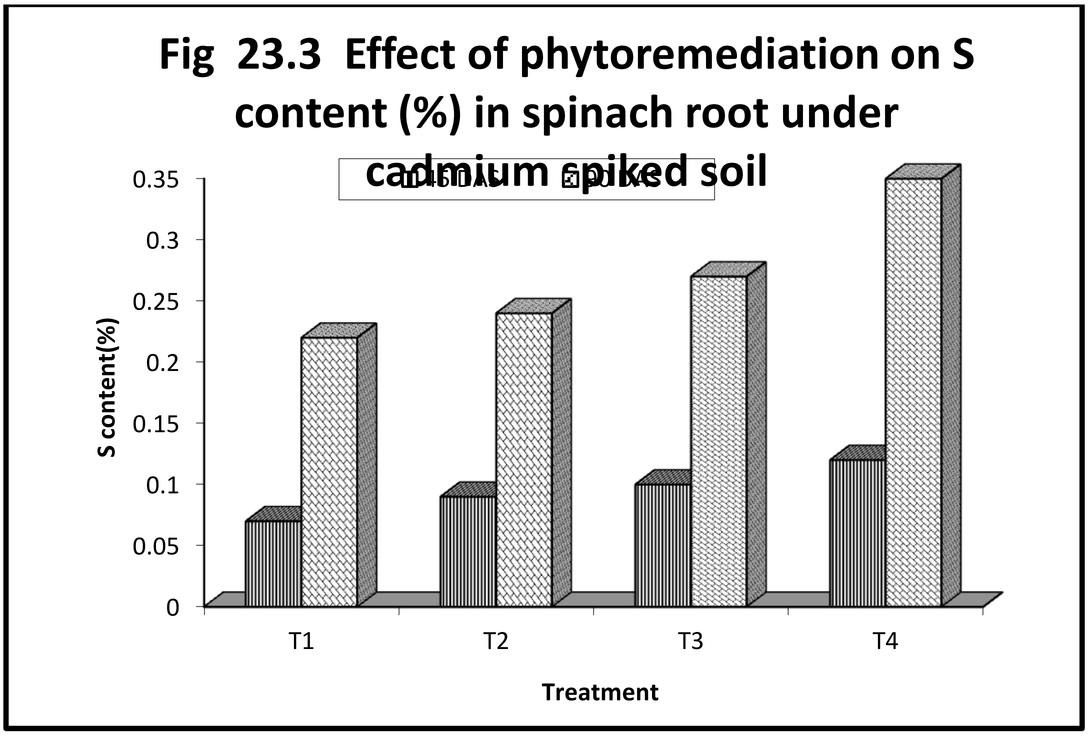


Fig 23.3 Effect of phytoremediation on S content (%) in spinach root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

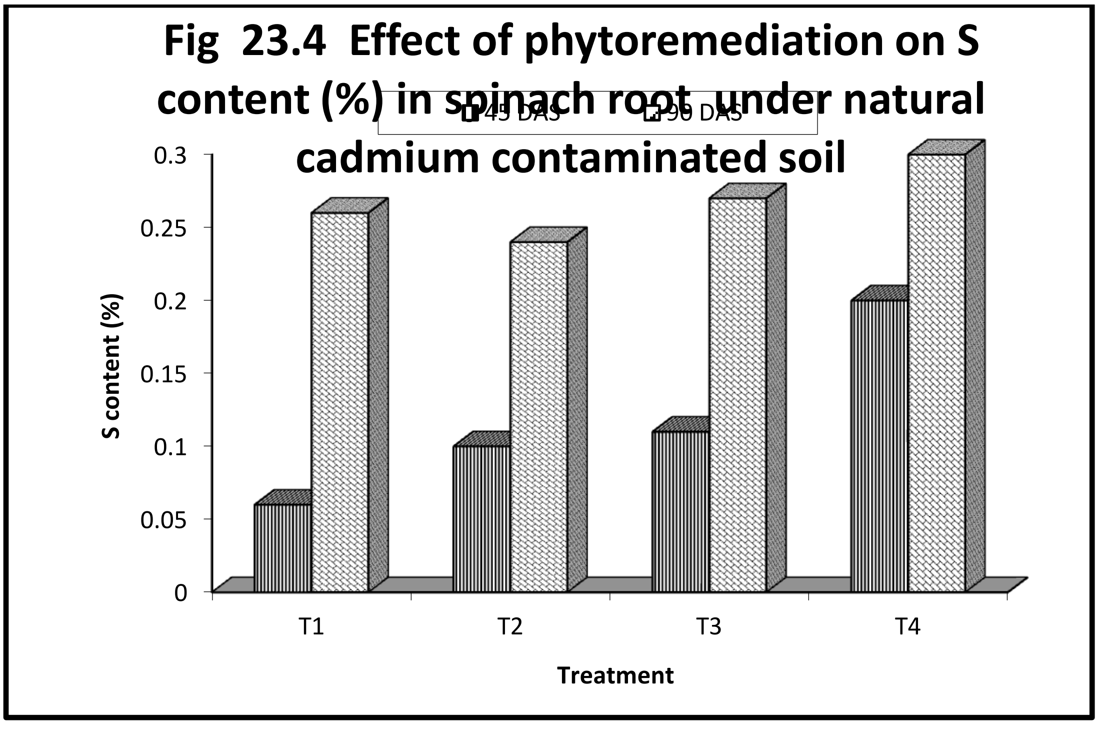


Fig 23.4 Effect of phytoremediation on S content (%) in spinach root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 24.1 Effect of phytoremediation on Cd content (ppm) in spinach plant under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**			
		45 DAS	**60 DAS**	**90 DAS**	**Mean**
T1	**RDF**	91.60	97.40	119.67	108.53
T2	**RDF+SSP**	96.47	98.20	107.20	102.70
T3	**RDF+AM**	88.47	96.60	115.07	105.83
T4	**RDF+SSP+AM**	87.67	110.33	117.87	114.10
Mean		91.05	100.63	114.95	
		SEm±	**CD at 5% level**		
Treatment (T)		4.72	14.16	**NS**	
Stage (S)		4.09	12.27	**SIG**	
Interaction (TxS)		8.18	24.53	**SIG**	

The effects of treatment had non-significant effect on Cd content in the spinach plant (Table, Fig 24.1 and Appendix 1.13). The stages of the crop growth had significant effect of Cd accumulation on the spinach plant. In 90 DAS (114.95 ppm) the plant had much the higher Cd content than the 60 DAS (100.63 ppm) and 45 DAS (91.05 ppm). In this study the interaction between growing stage and treatment due to Cd content AM+SSP addition had significant role on spinach plant Cd content in cadmium spiked soil. In 90 DAS among treatment the T1 (119.67 ppm) was found superior than other treatment combination with growing stage of spinach plant the Cd content decrease and which was found highest in T4 treatment.

Table 24.2 Effect of phytoremediation on Cd content (ppm) in spinach plant under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**			
		45 DAS	**60 DAS**	**90 DAS**	**Mean**
T1	**RDF**	1.40	1.47	2.53	2.00
T2	**RDF+SSP**	1.20	1.80	4.80	3.30
T3	**RDF+AM**	1.53	2.33	2.20	2.27
T4	**RDF+SSP+AM**	2.07	2.20	2.47	2.33
Mean		1.55	1.95	3.00	
		SEm±	**CD at 5% level**		
Treatment (T)		0.30	0.89	**NS**	
Stage (S)		0.26	0.77	**SIG**	
Interaction (TxS)		0.52	1.55	**SIG**	

The effects of treatment had non-significant effect on Cd content in the spinach plant (Table, Fig 24.2 and Appendix 1.14). The stages of the crop growth had significant effect of Cd content on the plant. In 90 DAS (3.00 ppm) the plant had much the higher Cd content than the 60 DAS (1.95 ppm) and 45 DAS (1.55 ppm). In this study the interaction between growing stage and treatment due to Cd content RDF+SSP addition had significant role on spinach plant Cd content in naturally cadmium contaminated soil. In 90 DAS among treatment the T2 (4.80 ppm) was found superior than other treatment combination with growing stage of spinach plant the Cd content decrease and which was found highest in T2 treatment.

Table 24.3 Effect of phytoremediation on Cd content (ppm) in spinach root under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	82.20	113.87	98.03
T2	**RDF+SSP**	85.13	117.67	101.40
T3	**RDF+AM**	88.53	115.13	101.83
T4	**RDF+SSP+AM**	93.47	104.67	99.07
Mean		87.33	112.83	
		SEm±	**CD at 5% level**	
Treatment (T)		6.72	20.16	NS
Stage (S)		4.76	14.26	SIG
Interaction (TxS)		8.24	24.69	NS

The effects of treatment had non-significant effect on Cd content in the spinach plant root (Table, Fig 24.3 and Appendix 1.15). The stages of the crop growth had significant effect on Cd content on the plant root. In 90 DAS (112.83 ppm) plant root had much the higher Cd content than the 45 DAS (87.33 ppm). There was found no significant role which the interaction of growing stage and treatment in the Cd content of in spinach root part.

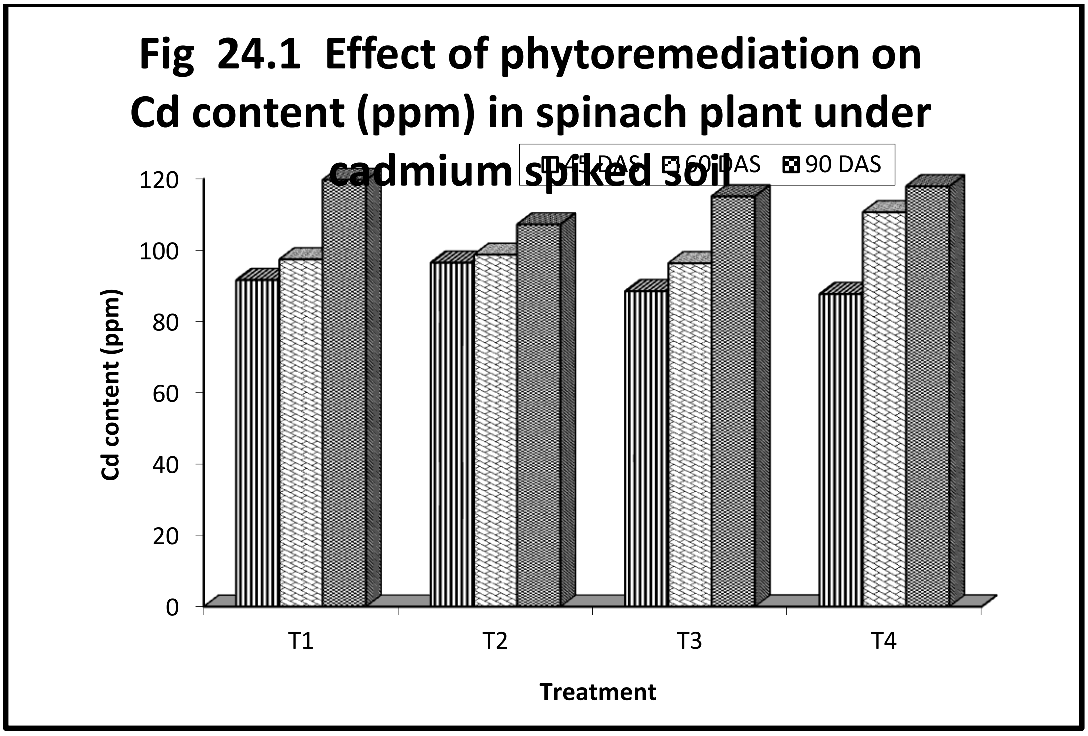


Fig 24.1 Effect of phytoremediation on Cd content (ppm) in spinach plant under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

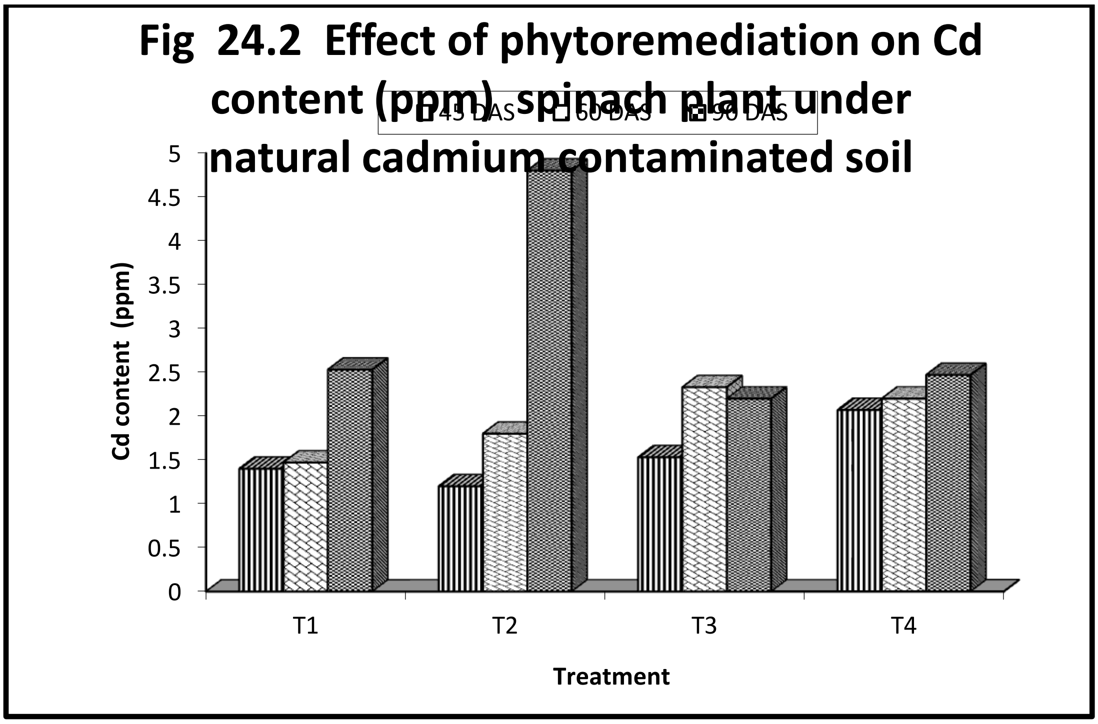


Fig 24.2 Effect of phytoremediation on Cd content (ppm) spinach plant under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 24.4 Effect of phytoremediation on Cd content (ppm) in spinach root under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.87	2.20	1.53
T2	**RDF+SSP**	0.80	2.87	1.83
T3	**RDF+AM**	0.93	2.87	1.90
T4	**RDF+SSP+AM**	1.47	3.27	2.37
Mean		1.02	2.80	
		SEm±	**CD at 5% level**	
Treatment (T)		0.45	1.35	NS
Stage (S)		0.32	0.96	SIG
Interaction (TxS)		0.55	1.66	NS

The effects of treatment had non-significant effect on Cd content in the spinach plant root (Table, Fig 24.4 and Appendix 1.16). The stages of the crop growth had significant effect on Cd content on the plant root. In 90 DAS (2.80 ppm) plant root had much the higher Cd content than the 45 DAS (1.02 ppm). The interaction effect between growing stage and treatment was observed in non-significant.

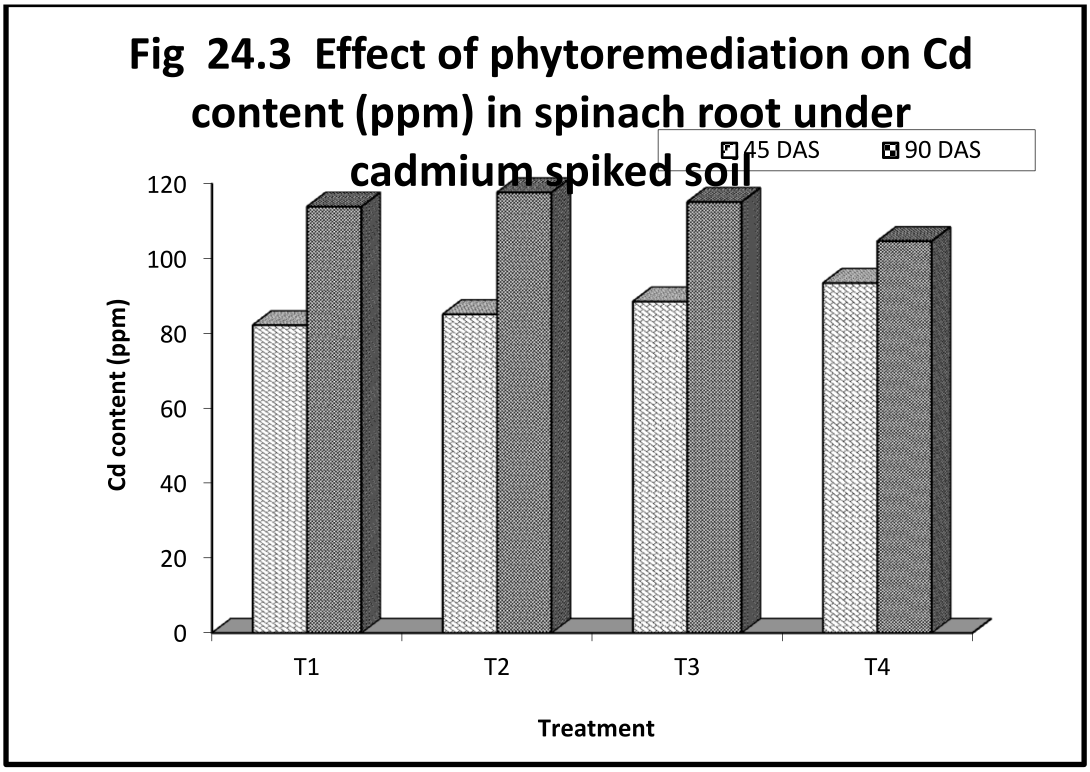


Fig 24.3 Effect of phytoremediation on Cd content (ppm) in spinach root under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

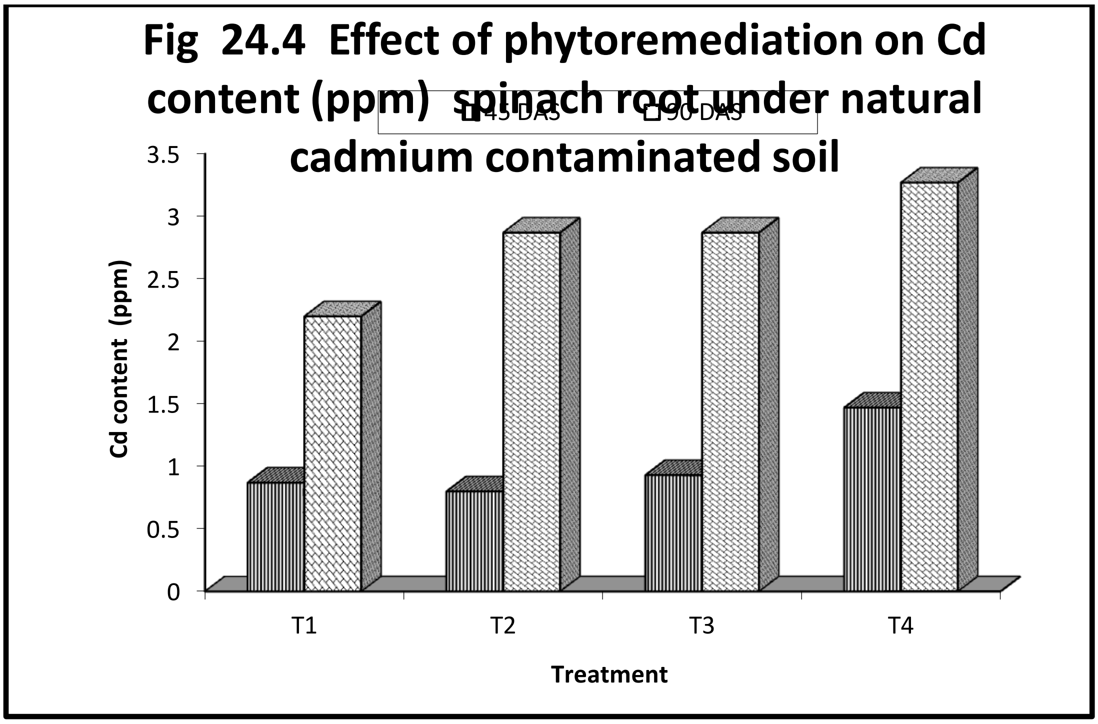


Fig 24.4 Effect of phytoremediation on Cd content (ppm) spinach root under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 25.1 Effect of phytoremediation on soil available N (kg ha^{-1}) in spinach crop under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	87.8	102.9	95.3
T2	**RDF+SSP**	99.9	117.1	108.5
T3	**RDF+AM**	117.1	137.1	127.1
T4	**RDF+SSP+AM**	138.0	146.8	142.4
Mean		110.7	126.0	
		SEm±	**CD at 5% level**	
Treatment (T)		3.95	11.85	**SIG**
Stage (S)		2.80	8.38	**SIG**
Interaction (TxS)		4.84	14.52	**NS**

The effects of treatment had significant effect on available N in the spinach which was found 127.1 kg ha^{-1} (T3) and 108.5 kg ha^{-1} (T2) respectively (Table, Fig 25.1 and Appendix 1.17). The T4 (RDF+AM+SSP) was found most prominent effect available N 142.4 kg ha^{-1} where as T1 (RDF) was found in the least available N (95.3 kg ha^{-1}) in the spinach. The stages of the crop growth had significant effect on available N on the spinach crop. In 90 DAS (126 kg ha^{-1}) the spinach had much the higher available N than the 45 DAS (110.7 kg ha^{-1}). The interaction effect between growing stage and treatment had non-significant in the available N in spinach crop.

Table 25.2 Effect of phytoremediation on soil available N (kg ha^{-1}) in spinach crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	118.7	129.2	124.0
T2	**RDF+SSP**	150.5	131.3	140.9
T3	**RDF+AM**	163.1	144.7	153.9
T4	**RDF+SSP+AM**	175.6	158.5	167.0
Mean		152.0	140.9	
		SEm±	**CD at 5% level**	
Treatment (T)		4.17	12.49	**SIG**
Stage (S)		2.95	8.83	**SIG**
Interaction (TxS)		5.10	15.30	**NS**

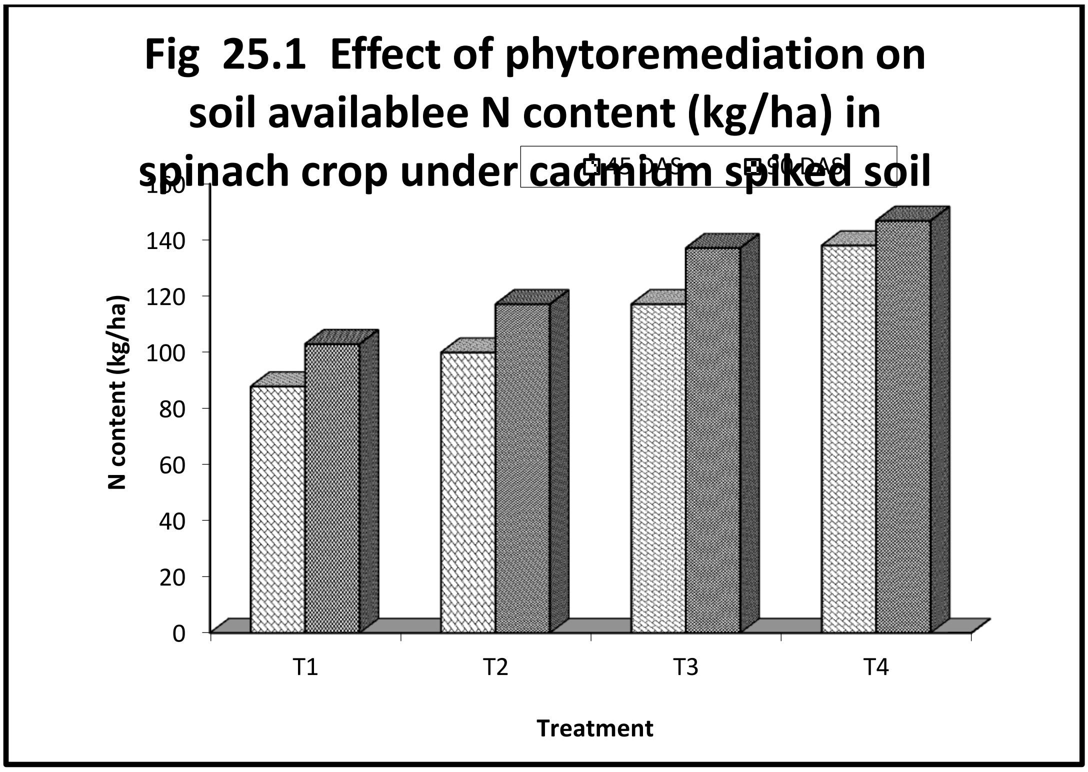


Fig 25.1 Effect of phytoremediation on soil availablee N content (kg/ha) in spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

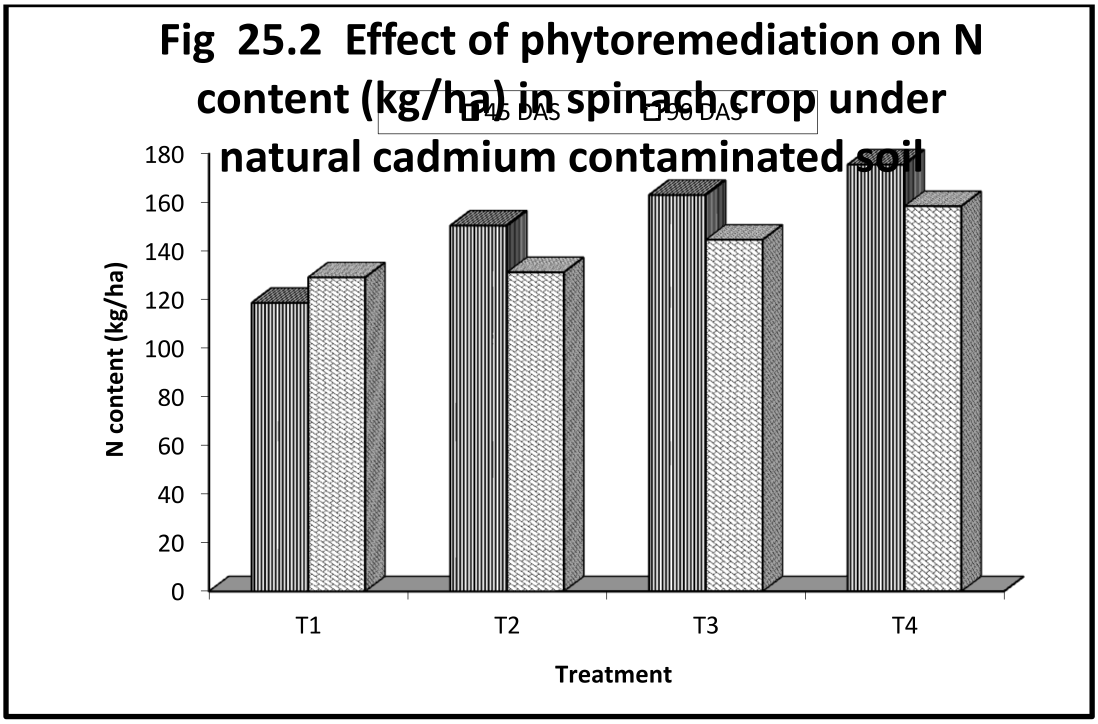


Fig 25.2 Effect of phytoremediation on N content (kg/ha) in spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on available N in the spinach crop which was observed 153.9 kg ha^{-1} (T3) and 140.9 kg ha^{-1} (T2) respectively (Table, Fig 25.2 and Appendix 1.18). The T4 (RDF+AM+SSP) was found most prominent effect available N (167 kg ha^{-1}) where as T1 (RDF) was found in the least content (124 kg ha^{-1}) in the spinach crop. The stages of the crop growth had significant effect on available N on the spinach crop. In 90 DAS (140.9 kg ha^{-1}) the spinach had much the lower available N than the 45 DAS (152 kg ha^{-1}). The interaction effect between growing stage and treatment was found in non-significant.

Table 26.1 Effect of phytoremediation on soil available P (kg ha^{-1}) in spinach crop under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	81.3	91.8	86.6
T2	**RDF+SSP**	94.1	107.5	100.8
T3	**RDF+AM**	100.6	115.3	107.9
T4	**RDF+SSP+AM**	114.3	130.4	122.4
Mean		97.6	111.3	
		SEm±	**CD at 5% level**	
Treatment (T)		3.45	10.35	**SIG**
Stage (S)		2.44	7.32	**SIG**
Interaction (TxS)		4.23	12.68	**NS**

The effects of treatment had significant effect on available P in the spinach which was found 107.9 kg ha^{-1} (T3) and 100.8 kg ha^{-1} (T2) respectively (Table, Fig 26.1 and Appendix 1.17). The T4 (RDF+AM+SSP) was found most prominent effect available P 122.4 kg ha^{-1} where as T1 (RDF) was found in the least available P (86.6 kg ha^{-1}) in the spinach. The stages of the crop growth had significant effect on available P on the spinach crop. In 90 DAS (111.3 kg ha^{-1}) spinach had much the higher available P than the 45 DAS (97.6 kg ha^{-1}). The interaction effect between growing stage and treatment was observed non-significant in the available P in spinach crop.

Table 26.2 Effect of phytoremediation on soil available P (kg ha^{-1}) in spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	101.0	98.7	99.9
T2	**RDF+SSP**	107.0	119.4	113.2
T3	**RDF+AM**	114.8	136.8	125.8
T4	**RDF+SSP+AM**	123.5	160.7	142.1
Mean		111.6	128.9	
		SEm±	**CD at 5% level**	
Treatment (T)		5.63	16.88	**SIG**
Stage (S)		3.98	11.93	**SIG**
Interaction (TxS)		6.89	20.67	**NS**

The effects of treatment had significant effect on available P in the spinach crop which was observed 125.8 kg ha^{-1} (T3) and 113.2 kg ha^{-1} (T2) respectively (Table, Fig 26.2 and Appendix 1.18). The T4 (RDF+AM+SSP) was found most prominent effect available P (142 kg ha^{-1}) where as T1 (RDF) was found in the least P content (99.9 kg ha^{-1}) in the spinach crop. The stages of the crop growth had significant effect on available P on the spinach crop. In 90 DAS (128.9 kg ha^{-1}) the spinach had much the maximum available P than the 45 DAS (111.6 kg ha^{-1}). The interaction effect between growing stage and treatment was found in non-significant.

Table 27.1 Effect of phytoremediation on soil available K (kg ha^{-1}) in spinach crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	354.7	324.1	339.4
T2	**RDF+SSP**	380.8	334.5	357.7
T3	**RDF+AM**	390.1	349.1	369.6
T4	**RDF+SSP+AM**	399.1	357.7	378.4
Mean		381.2	341.4	
		SEm±	**CD at 5% level**	
Treatment (T)		10.07	30.18	**NS**
Stage (S)		7.12	21.34	**SIG**
Interaction (TxS)		12.33	36.96	**NS**

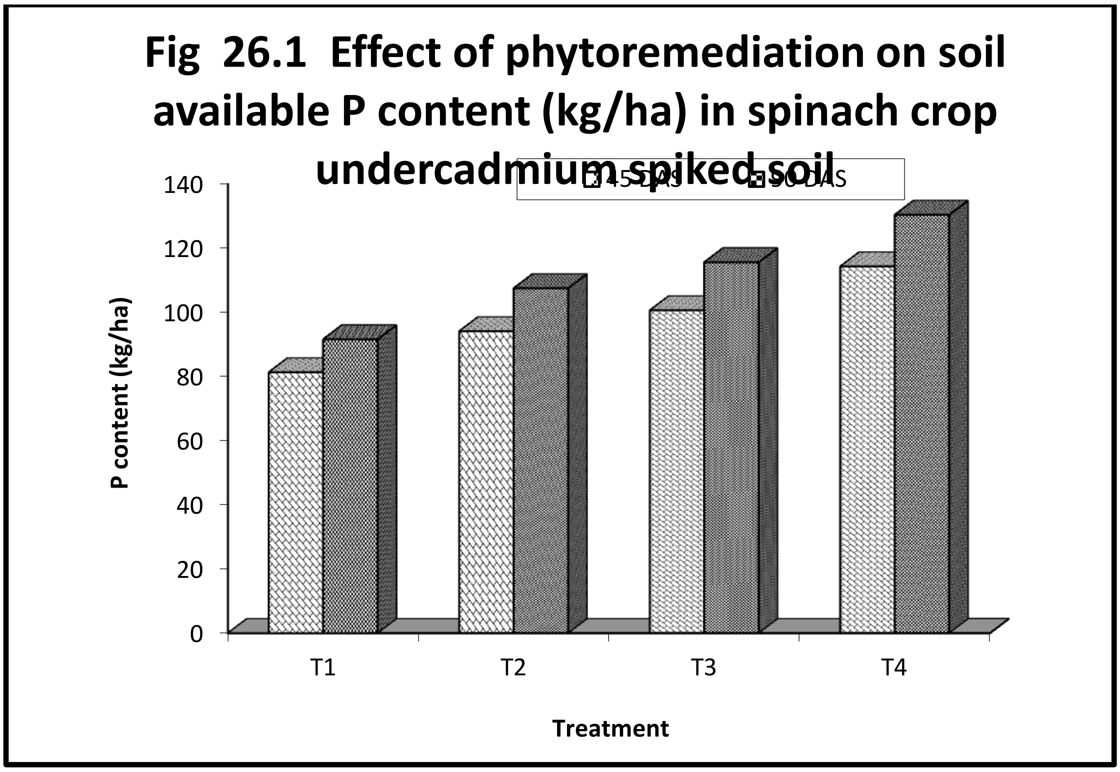


Fig 26.1 Effect of phytoremediation on soil available P content (kg/ha) in spinach crop undercadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

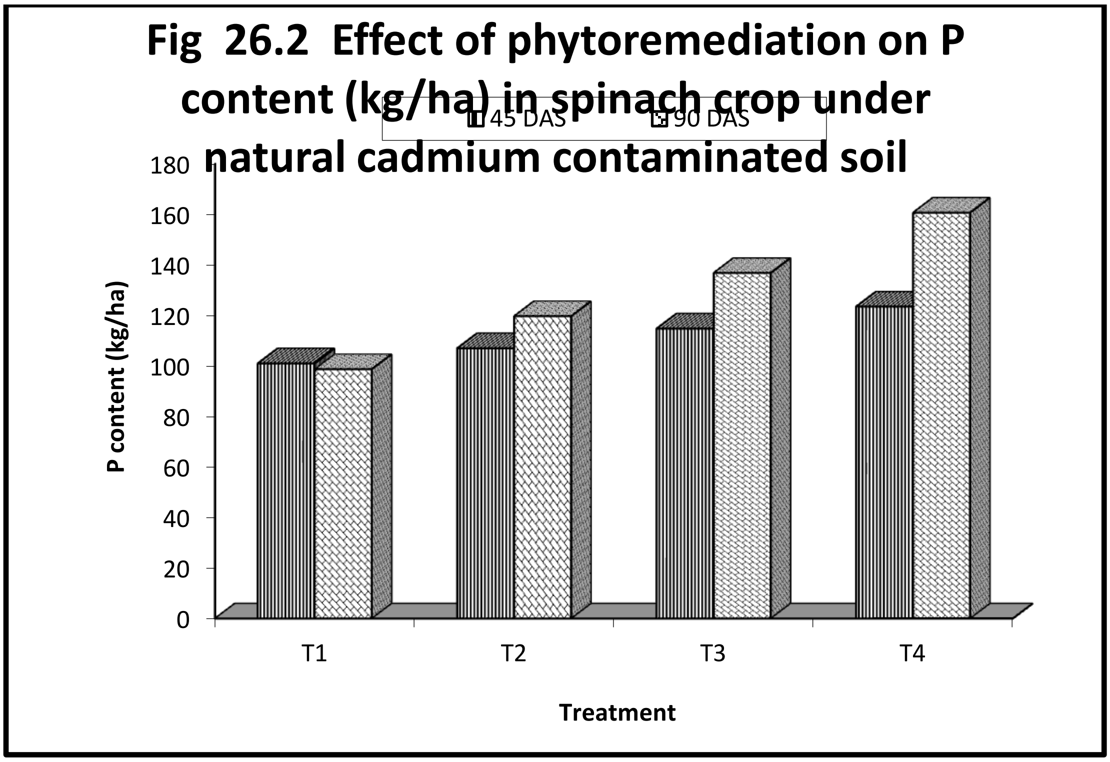


Fig 26.2 Effect of phytoremediation on P content (kg/ha) in spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on available K in the spinach (Table, Fig 27.1 and Appendix 1.17). The stages of the crop growth had significant effect on available K on the spinach crop. In 90 DAS (341.4 kg ha^{-1}) the spinach had much the lower available K than the 45 DAS (381.2 kg ha^{-1}). The interaction effect between growing stage and treatment was observed non-significant in the available K in spinach crop.

Table 27.2 Effect of phytoremediation on soil available K (kg ha^{-1}) in spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	391.3	334.9	363.1
T2	**RDF+SSP**	399.8	363.3	381.5
T3	**RDF+AM**	405.8	377.4	391.6
T4	**RDF+SSP+AM**	419.3	387.5	403.4
Mean		404.1	365.8	
		SEm±	**CD at 5% level**	
Treatment (T)		9.94	29.81	**NS**
Stage (S)		7.03	21.08	**SIG**
Interaction (TxS)		12.18	36.51	**NS**

The effects of treatment had non-significant effect on available K in the spinach crop (Table, Fig 27.2 and Appendix 1.18). The stages of the crop growth had significant effect on available K on the spinach crop. In 90 DAS (365.8 kg ha^{-1}) the spinach was much the lower available K than the 45 DAS (404.1 kg ha^{-1}). The interaction effect between growing stage and treatment was found in non-significant.

Table 28.1 Effect of phytoremediation on soil available S (mg kg^{-1}) in spinach crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.049	0.061	0.055
T2	**RDF+SSP**	0.054	0.075	0.064
T3	**RDF+AM**	0.062	0.055	0.058
T4	**RDF+SSP+AM**	0.089	0.071	0.080
Mean		0.064	0.065	
		SEm±	**CD at 5% level**	
Treatment (T)		0.004	0.012	**SIG**
Stage (S)		0.003	0.008	**NS**
Interaction (TxS)		0.005	0.014	**SIG**

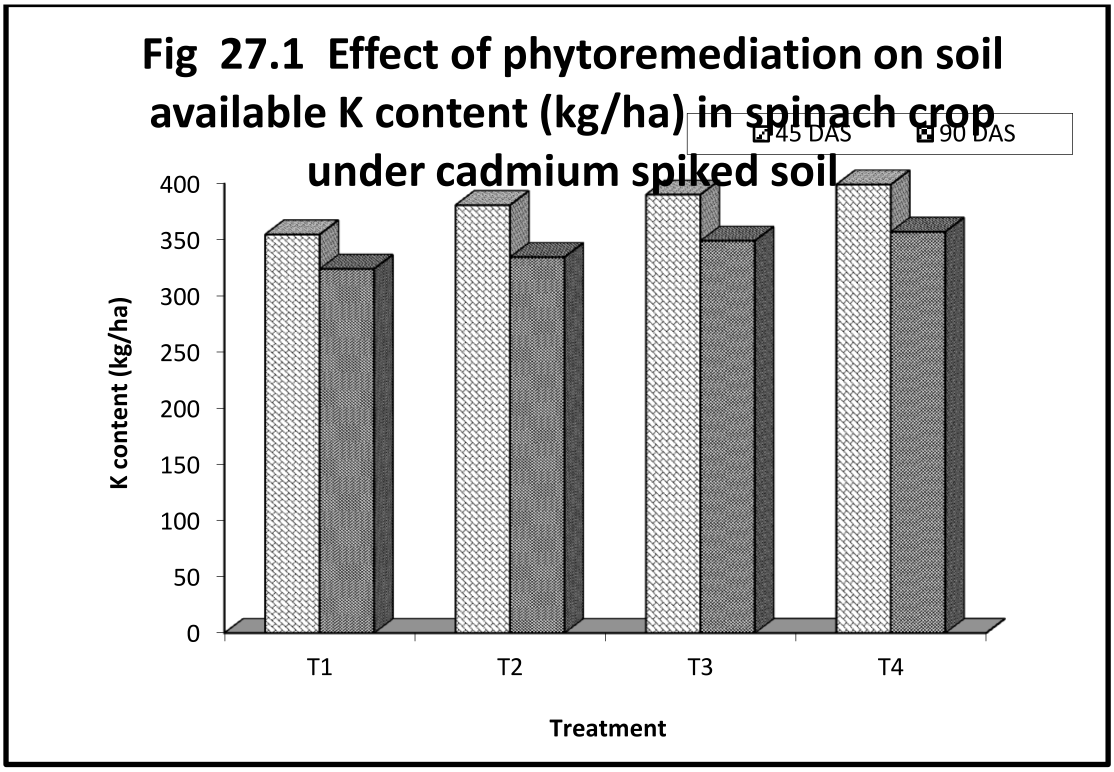


Fig 27.1 Effect of phytoremediation on soil available K content (kg/ha) in spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

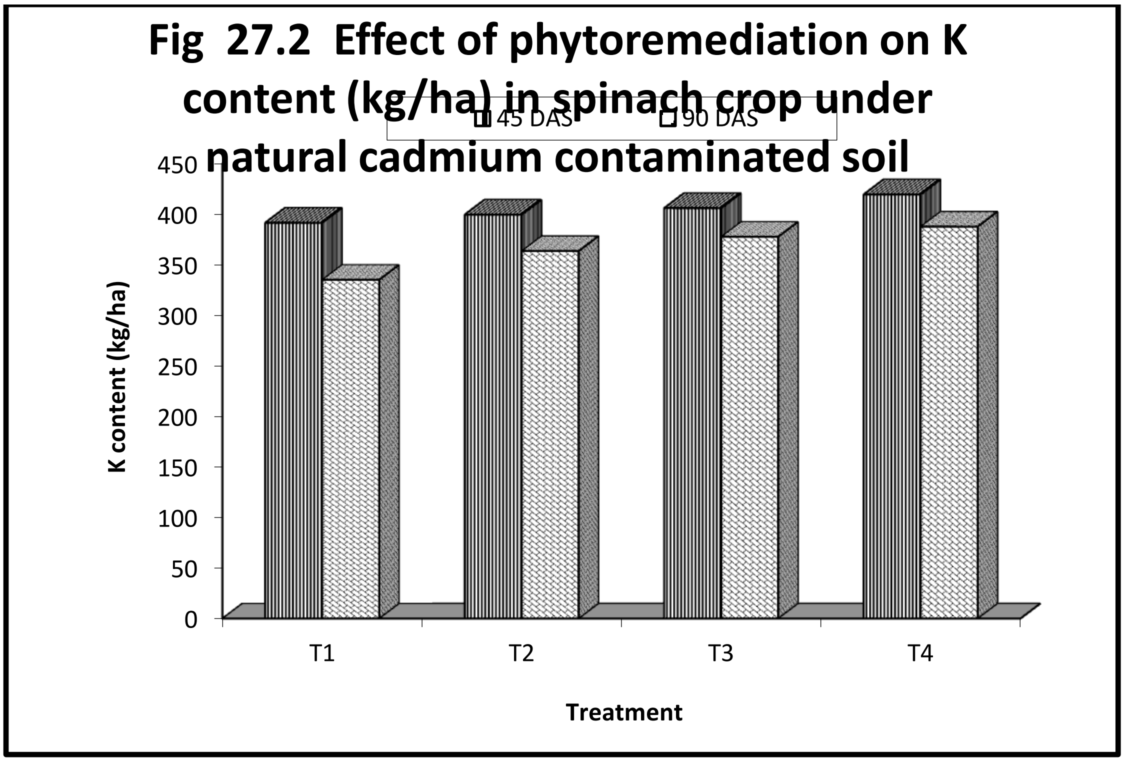


Fig 27.2 Effect of phytoremediation on K content (kg/ha) in spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on available S in the spinach which was found 0.058 mg kg^{-1} (T3) and 0.064 mg kg^{-1} (T2) respectively (Table, Fig 28.1 and Appendix 1.17). The T4 (RDF+AM+SSP) was found most prominent effect available S 0.080 mg kg^{-1} where as T1 (RDF) was observed in the least available S (0.055 mg kg^{-1}) in the spinach. The stages of the crop growth had non-significant effect on available S on the spinach crop. The interaction effect between growing stage and treatment was significant role in the S in spinach crop.

Table 28.2 Effect of phytoremediation on soil available S (mg kg^{-1}) in spinach crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.081	0.047	0.064
T2	**RDF+SSP**	0.097	0.057	0.077
T3	**RDF+AM**	0.109	0.065	0.087
T4	**RDF+SSP+AM**	0.149	0.074	0.111
Mean		0.109	0.061	
		SEm±	**CD at 5% level**	
Treatment (T)		0.013	0.038	NS
Stage (S)		0.009	0.027	SIG
Interaction (TxS)		0.015	0.046	NS

The effects of treatment had non-significant effect on available S in the spinach crop (Table, Fig 28.2 and Appendix 1.18). The stages of the crop growth had significant effect on available S on the spinach crop. In 90 DAS (0.061 mg kg^{-1}) the spinach had much the higher available S than the 45 DAS (0.109 mg kg^{-1}). The interaction effect between growing stage and treatment was found in non-significant.

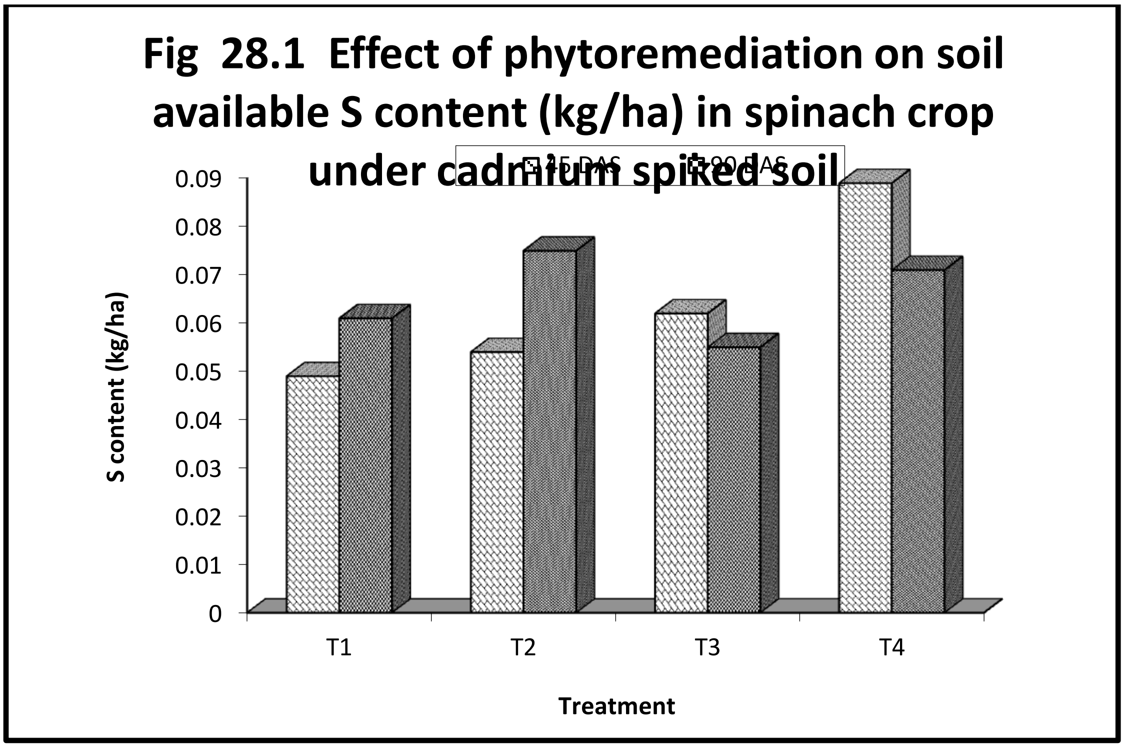


Fig 28.1 Effect of phytoremediation on soil available S content (kg/ha) in spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

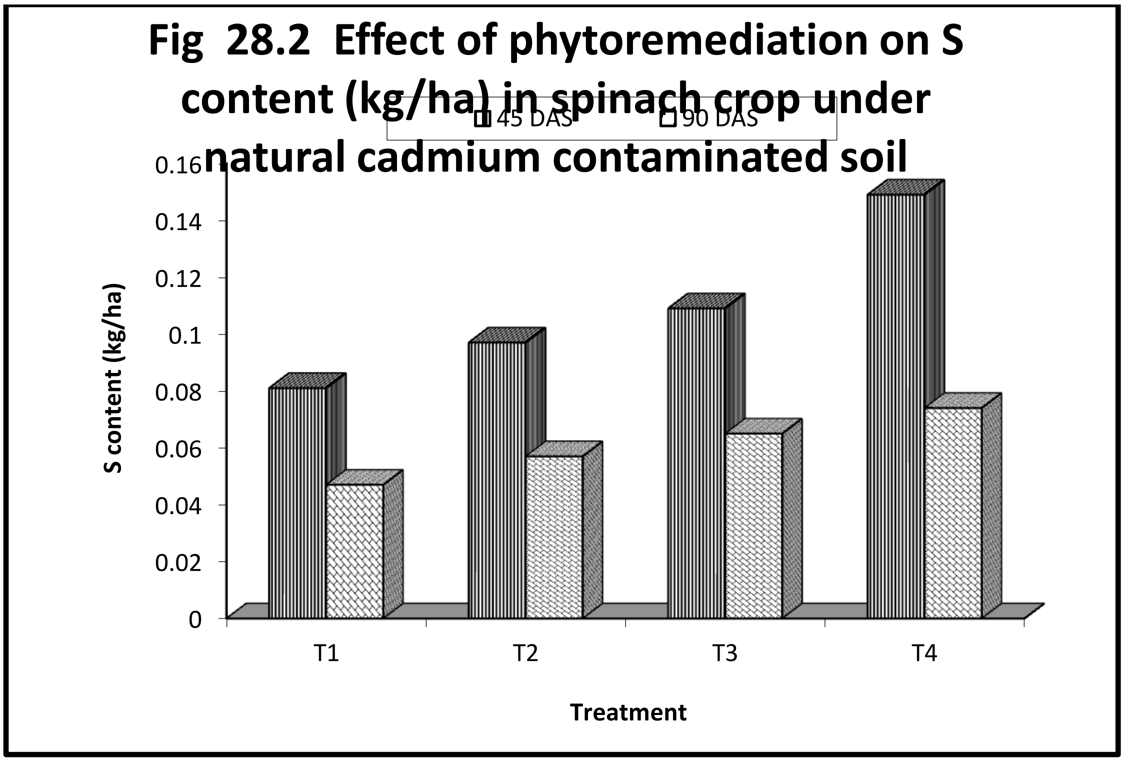


Fig 28.2 Effect of phytoremediation on S content (kg/ha) in spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 29.1 Effect of phytoremediation on soil Cd (ppm) in spinach crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	72.25	70.55	71.40
T2	**RDF+SSP**	45.75	43.71	44.73
T3	**RDF+AM**	75.70	72.56	74.13
T4	**RDF+SSP+AM**	80.03	75.65	77.84
Mean		68.43	65.62	
		SEm±	**CD at 5% level**	
Treatment (T)		0.75	2.25	**SIG**
Stage (S)		0.53	1.59	**SIG**
Interaction (TxS)		0.92	2.76	**NS**

The effects of treatment had significant effect on Cd in the spinach which was found 74.13 ppm (T3) and 71.40 ppm (T1) respectively (Table, Fig 29.1 and Appendix 1.17). The T4 (RDF+AM+SSP) was found most prominent effect Cd 77.84 ppm M where as T2 (RDF+SSP) was observed in the least Cd (44.73 ppm) in the spinach. The stages of the crop growth had significant effect on Cd on the spinach crop. In 45 DAS (68.43 ppm) the spinach had much the higher Cd than the 90 DAS (65.62 ppm). The interaction effect between growing stage and treatment was showed non-significant role in the Cd in spinach crop.

Table 29.2 Effect of phytoremediation on soil Cd (ppm) in spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.43	0.33	0.38
T2	**RDF+SSP**	0.34	0.28	0.31
T3	**RDF+AM**	0.42	0.35	0.39
T4	**RDF+SSP+AM**	0.37	0.33	0.35
Mean		0.39	0.32	
		SEm±	**CD at 5% level**	
Treatment (T)		0.018	0.053	**SIG**
Stage (S)		0.013	0.038	**SIG**
Interaction (TxS)		0.022	0.065	**NS**

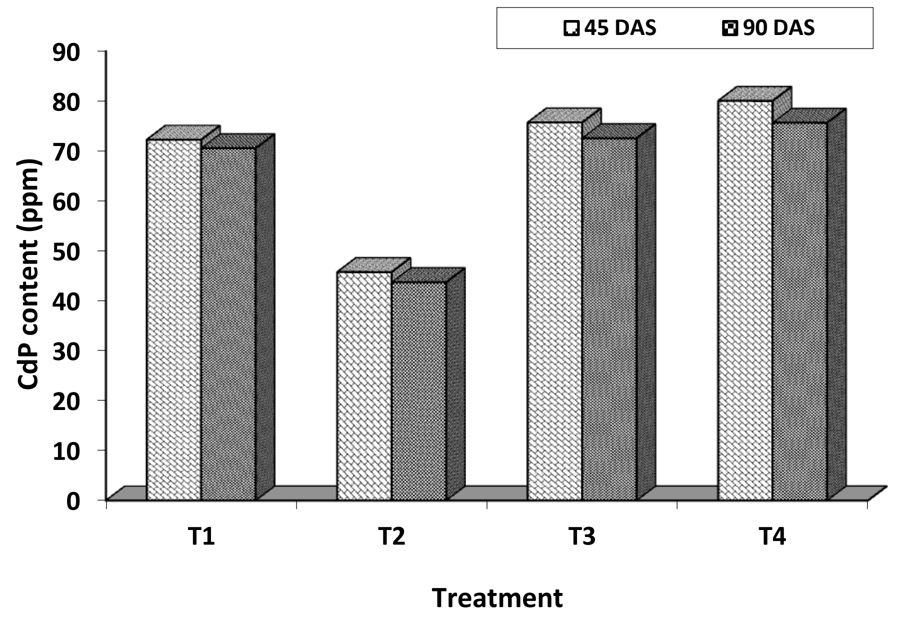


Fig 29.1 Effect of phytoremediation on soil Cd content (ppm) in spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate

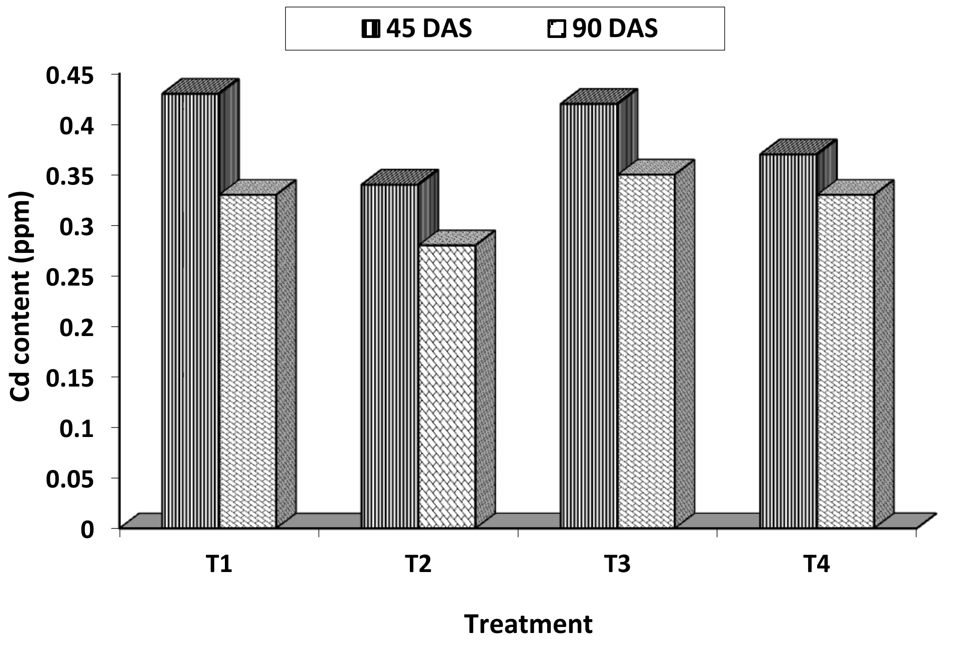


Fig 29.2 Effect of phytoremediation on Cd content (ppm) in spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had non-significant effect on Cd in the spinach crop (Table, Fig 29.2 and Appendix 1.18). The stages of the crop growth had significant effect of Cd on the spinach crop. In 45 DAS (0.39 ppm) the spinach had much the higher Cd than the 90 DAS (0.32 ppm). The interaction effect between growing stage and treatment was found in non-significant.

Table 30.1 Effect of phytoremediation on dehydrogenase activity (*μg* TPF g soil^{-1} 24h^{-1}) in spinach crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	27.3	85.8	56.5
T2	**RDF+SSP**	37.8	89.4	63.6
T3	**RDF+AM**	47.3	103.5	75.4
T4	**RDF+SSP+AM**	62.8	109.8	86.3
Mean		43.8	97.1	
		SEm±	**CD at 5% level**	
Treatment (T)		13.38	40.10	**NS**
Stage (S)		9.46	28.36	**SIG**
Interaction (TxS)		16.38	49.12	**NS**

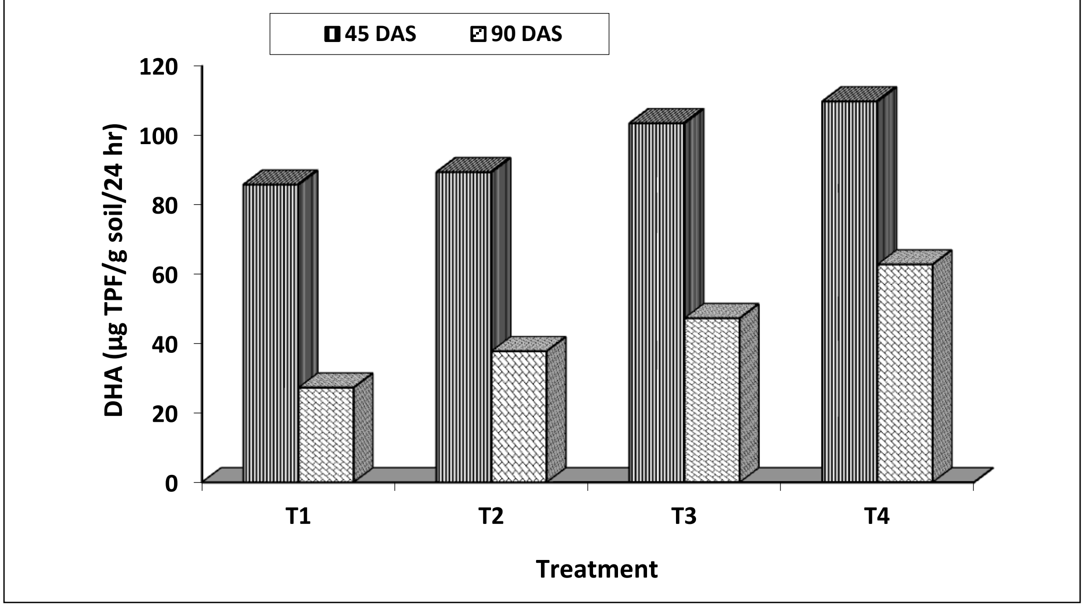
The effects of treatment had non-significant effect on DHA in the spinach (Table, Fig 30.1 and Appendix 1.19). The stages of the crop growth had significant effect of DHA on the spinach crop. In 45 DAS (43.8 *μg* TPF g soil^{-1} 24h^{-1}) the spinach had much the lower DHA than the 90 DAS (97.1 *μg* TPF g soil^{-1} 24h^{-1}). The interaction effect between growing stage and treatment was no role in the DHA in spinach crop.

Table 30.2 Effect of phytoremediation on dehydrogenase activity (*μg* TPF g soil^{-1} 24h^{-1}) in spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	28.9	131.1	80.0
T2	**RDF+SSP**	36.2	141.7	88.9
T3	**RDF+AM**	53.6	143.6	98.6
T4	**RDF+SSP+AM**	73.6	153.8	113.7
Mean		48.1	142.6	
		SEm±	**CD at 5% level**	
Treatment (T)		23.76	71.22	**NS**
Stage (S)		16.80	50.36	**SIG**
Interaction (TxS)		29.10	87.23	**NS**

The effects of treatment had non-significant effect on DHA in the spinach crop (Table, Fig 30.2 and Appendix 1.20). The stages of the crop growth had significant

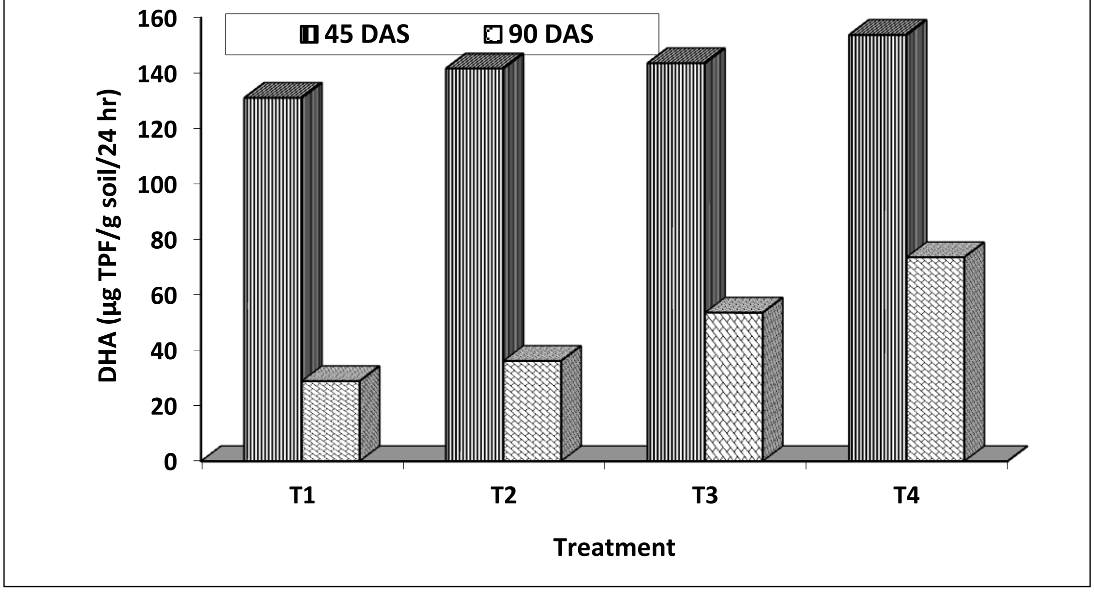
Fig 30.1 Effect of phytoremediation on DHA (µg TPF/g soil/24 hr) in spinach crop under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 30.2 Effect of phytoremediation on DHA (µg TPF/g soil/24 hr) in spinach crop under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

effect of DHA on the spinach crop. In 45 DAS (48.1 *µg* TPF g soil^{-1} 24h^{-1}) the spinach was much the minimum DHA than the 90 DAS (142.6 *µg* TPF g soil^{-1} 24h^{-1}). The interaction effect between growing stage and treatment was found in non-significant.

Table 31.1 Effect of phytoremediation on fluorescein di-acetate (*µg* Fluorescein g^{-1}) in spinach crop under cadmium spiked soil

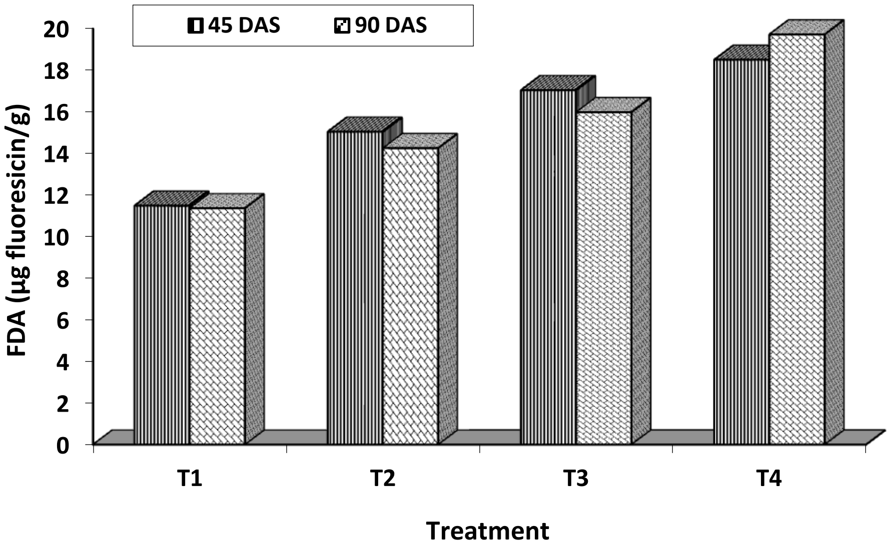
Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	11.37	11.49	11.43
T2	**RDF+SSP**	14.26	15.05	14.65
T3	**RDF+AM**	15.99	17.05	16.52
T4	**RDF+SSP+AM**	19.73	18.51	19.12
Mean		15.30	15.50	
		SEm±	**CD at 5% level**	
Treatment (T)		0.23	0.68	**SIG**
Stage (S)		0.16	0.48	**NS**
Interaction (TxS)		0.28	0.83	**SIG**

The effects of treatment had significant effect on FDA in the spinach which was found 16.52 *µg* fluorescein g^{-1} (T3) and 14.65 *µg* fluorescein g^{-1} (T2) respectively (Table, Fig 31.1 and Appendix 1.19). The T4 (RDF+AM+SSP) was found most prominent effect of FDA 19.12 *µg* fluorescein g^{-1} where as T1 (RDF) was observed in the least FDA 11.43 *µg* fluorescein g^{-1} in the spinach crop. The stages of the crop growth had non-significant effect of FDA on the spinach crop. The interaction effect between growing stage and treatment had significant role in the FDA in spinach crop.

Table 31.2 Effect of phytoremediation on fluorescein di-acetate (*µg* Fluorescein g^{-1}) in spinach crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	16.78	14.56	15.67
T2	**RDF+SSP**	17.48	23.14	20.31
T3	**RDF+AM**	18.51	25.26	21.89
T4	**RDF+SSP+AM**	20.73	28.94	24.84
Mean		18.4	23.0	
		SEm±	**CD at 5% level**	
Treatment (T)		1.53	4.59	**SIG**
Stage (S)		1.08	3.24	**SIG**
Interaction (TxS)		1.87	5.62	**NS**

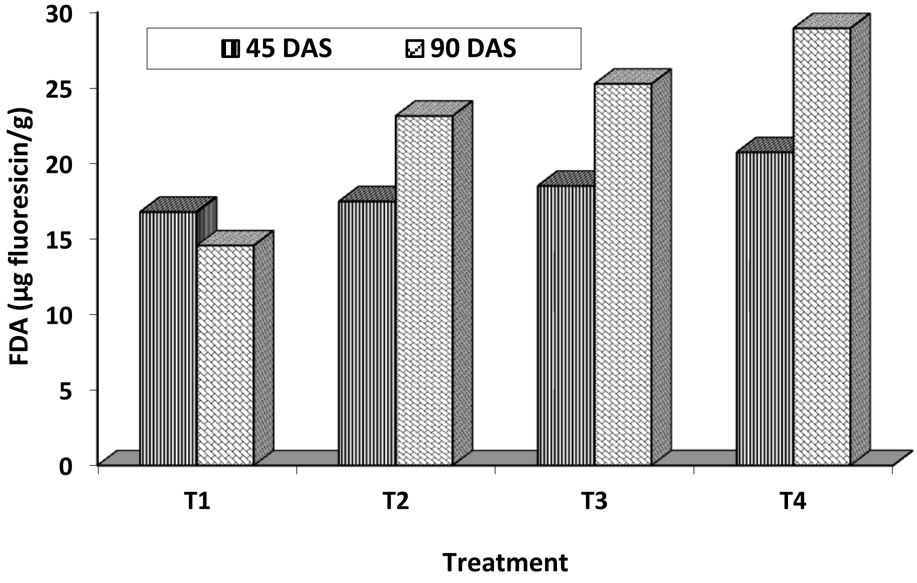
Fig 31.1 Effect of phytoremediation on fluorescein di-acetate (µg fluorescein/g) in mustard crop under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 31.2 Effect of phytoremediation on fluorescein di-acetate (µg fluorescein/g) in mustard crop under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on FDA in the spinach crop which was observed 21.89 *µg* fluorescein g^{-1} (T3) and 20.31 *µg* fluorescein g^{-1} (T2) respectively (Table, Fig 31.2 and Appendix 1.20). The T4 (RDF+AM+SSP) was found most prominent effect FDA (24.84 *µg* fluorescein g^{-1}) where as T1 (RDF) was found in the least content (15.67 *µg* fluorescein g^{-1}) in the spinach crop. The stages of the crop growth had significant effect of FDA µg on the spinach crop. In 90 DAS (23 *µg* fluorescein g^{-1}) the spinach was much the higher FDA than the 45 DAS (18.4 *µg* fluorescein g^{-1}). The interaction effect between growing stage and treatment was found in non-significant.

Table 32.1 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in spinach crop under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.035	0.031	0.033
T2	**RDF+SSP**	0.044	0.049	0.047
T3	**RDF+AM**	0.057	0.055	0.056
T4	**RDF+SSP+AM**	0.065	0.083	0.074
Mean		0.050	0.055	
		SEm±	**CD at 5% level**	
Treatment (T)		0.002	0.007	**SIG**
Stage (S)		0.002	0.005	**NS**
Interaction (TxS)		0.003	0.009	**SIG**

The effects of treatment had significant effect on soil respiration in the spinach which was found 0.056 mg CO_2-C g^{-1} soil d^{-1} (T3) and 0.047 mg CO_2-C g^{-1} soil d^{-1} (T2) respectively (Table, Fig 32.1 and Appendix 1.19). The T4 (RDF+AM+SSP) was found most prominent effect of soil respiration 0.074 mg CO_2-C g^{-1} soil d^{-1} where as T1 (RDF) was found in the least soil respiration 0.033 mg CO_2-C g^{-1} soil d^{-1} in the spinach crop. The stages of the crop growth had non-significant effect of soil respiration on the spinach crop. The interaction effect between growing stage and treatment was found significant role in the soil respiration in spinach crop.

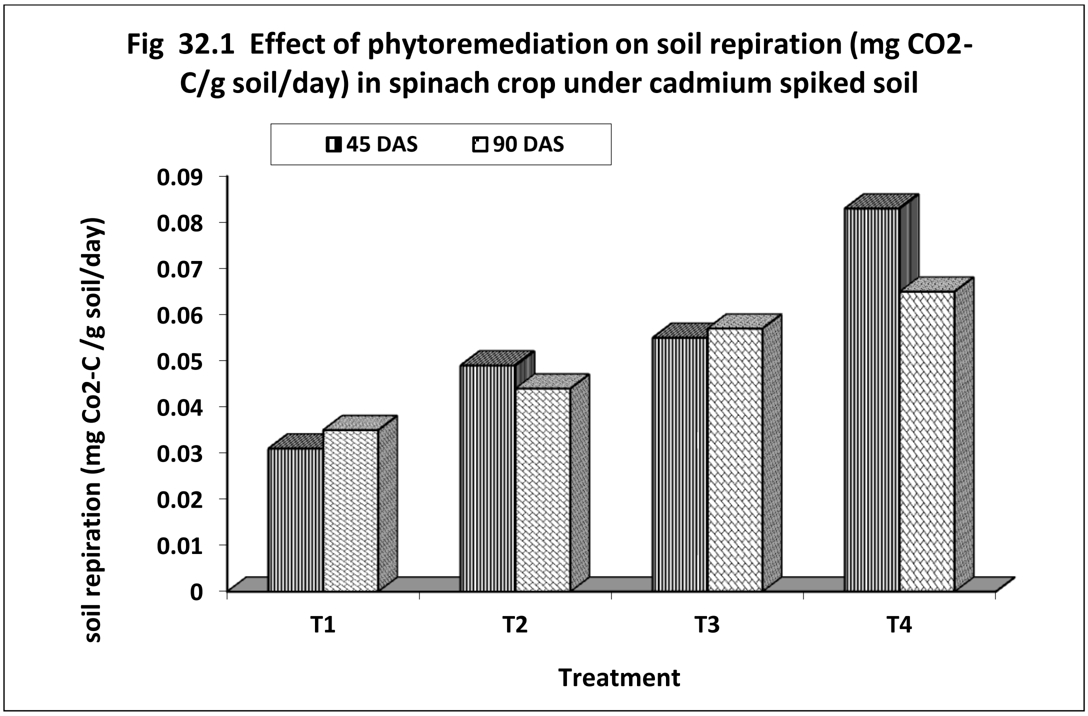


Fig 32.1 Effect of phytoremediation on soil repiration (mg CO2-C/g soil/day) in spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

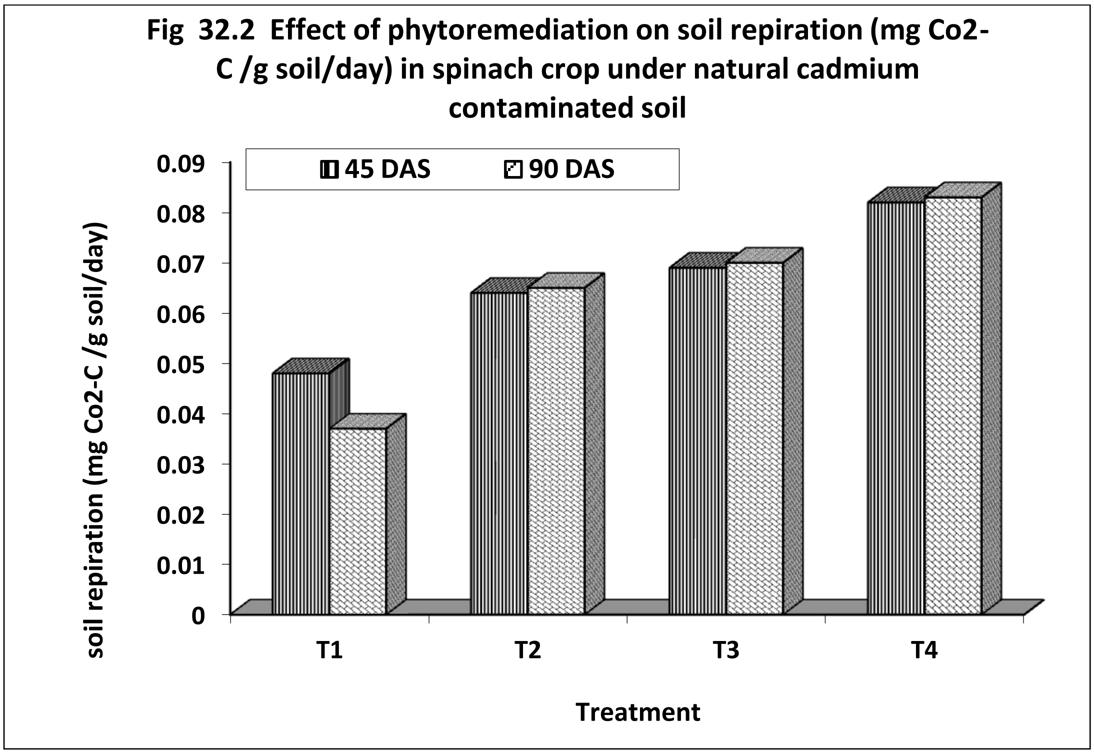


Fig 32.2 Effect of phytoremediation on soil repiration (mg Co2-C /g soil/day) in spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 32.2 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	90 DAS	Mean
T1	**RDF**	0.037	0.048	0.043
T2	**RDF+SSP**	0.065	0.064	0.065
T3	**RDF+AM**	0.070	0.069	0.070
T4	**RDF+SSP+AM**	0.083	0.082	0.083
Mean		0.064	0.066	
		SEm±	**CD at 5% level**	
Treatment (T)		0.001	0.004	**SIG**
Stage (S)		0.001	0.003	**NS**
Interaction (TxS)		0.002	0.005	**SIG**

The effects of treatment had significant effect on soil respiration in the spinach crop which was observed 0.070 mg CO_2-C g^{-1} soil d^{-1} (T3) and 0.065 mg CO_2-C g^{-1} soil d^{-1} (T2) respectively (Table, Fig 32.2 and Appendix 1.20). The T4 (RDF+AM+SSP) was found most prominent effect soil respiration (0.083 mg CO_2-C g^{-1} soil d^{-1}) where as T1 (RDF) was showed in the least content (0.043 mg CO_2-C g^{-1} soil d^{-1}) in the spinach crop. The stages of the crop growth had non-significant effect of soil respiration on the spinach crop. The interaction effect between growing stage and treatment was found in significant.

Table 33.1 Effect of phytoremediation on soil microbial biomass carbon (SMBC) in spinach crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	90 DAS	Mean
T1	**RDF**	0.74	0.98	0.86
T2	**RDF+SSP**	0.78	1.02	0.90
T3	**RDF+AM**	0.82	1.08	0.95
T4	**RDF+SSP+AM**	0.84	1.13	0.99
Mean		0.80	1.05	
		SEm±	**CD at 5% level**	
Treatment (T)		0.064	0.193	**NS**
Stage (S)		0.046	0.137	**SIG**

Interaction (TxS)	0.079	0.236	**NS**

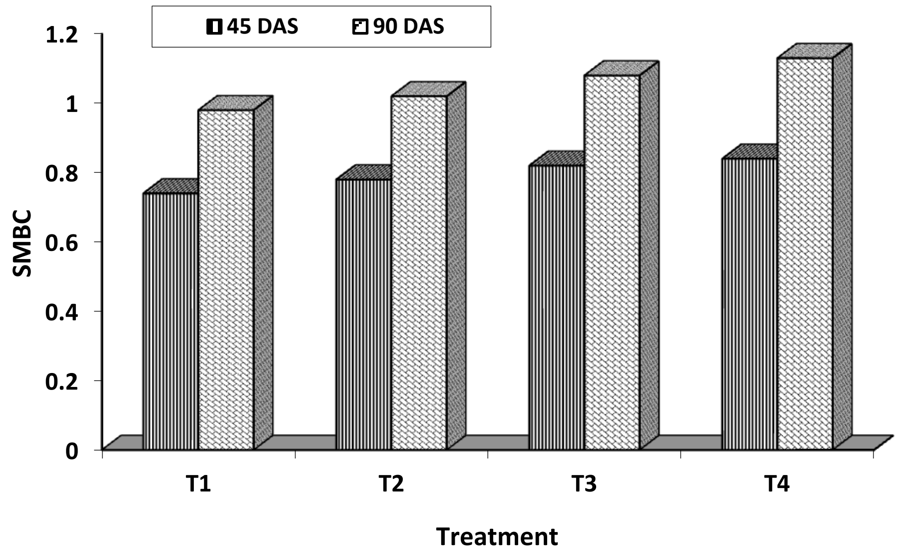
The effects of treatment had non-significant effect on SMBC in the spinach (Table, Fig 33.1 and Appendix 1.19). The stages of the crop growth had significant effect of SMBC on the spinach crop. In 90 DAS (1.05) the spinach had much the higher SMBC than the 45 DAS (0.80). The interaction effect between growing stage and treatment had non-significant role in the SMBC in spinach crop.

Table 33.2 Effect of phytoremediation on soil microbial biomass carbon (SMBC) in spinach crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	0.76	1.22	0.99
T2	**RDF+SSP**	0.78	1.24	1.01
T3	**RDF+AM**	0.84	1.44	1.14
T4	**RDF+SSP+AM**	0.87	1.49	1.18
Mean		0.81	1.35	
		SEm±	**CD at 5% level**	
Treatment (T)		0.135	0.406	**NS**
Stage (S)		0.096	0.287	**SIG**
Interaction (TxS)		0.166	0.497	**NS**

The effects of treatment had non-significant effect on SMBC in the spinach crop (Table, Fig 33.2 and Appendix 1.20). The stages of the crop growth had significant effect of SMBC on the spinach crop. In 90 DAS (1.35) the spinach was much the higher SMBC than the 45 DAS (0.81). The interaction effect between growing stage and treatment was found in non-significant.

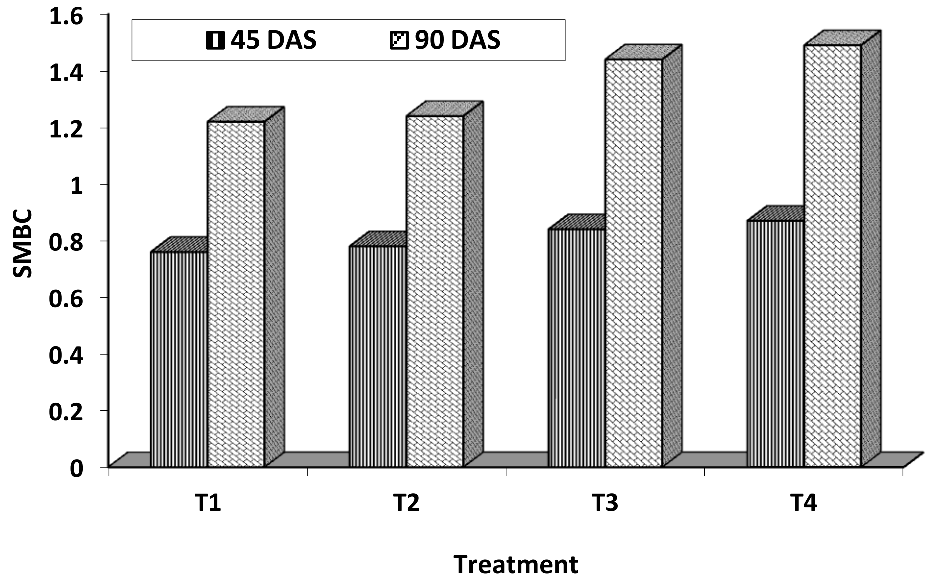
Fig 33.1 Effect of phytoremediation on soil biomass carbon (SMBC) in spinach crop under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 33.2 Effect of phytoremediation on soil biomass carbon (SMBC) in spinach crop under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 34.1 Effect of phytoremediation on glomalin (mg kg^{-1}) content in soil during spinach crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	30.59	33.76	32.17
T2	**RDF+SSP**	31.72	34.60	33.16
T3	**RDF+AM**	22.88	35.38	35.13
T4	**RDF+SSP+AM**	33.30	43.23	38.26
Mean		29.62	36.74	
		SEm±	**CD at 5% level**	
Treatment (T)		2.07	6.19	**SIG**
Stage (S)		1.46	4.38	**SIG**
Interaction (TxS)		2.53	7.58	**NS**

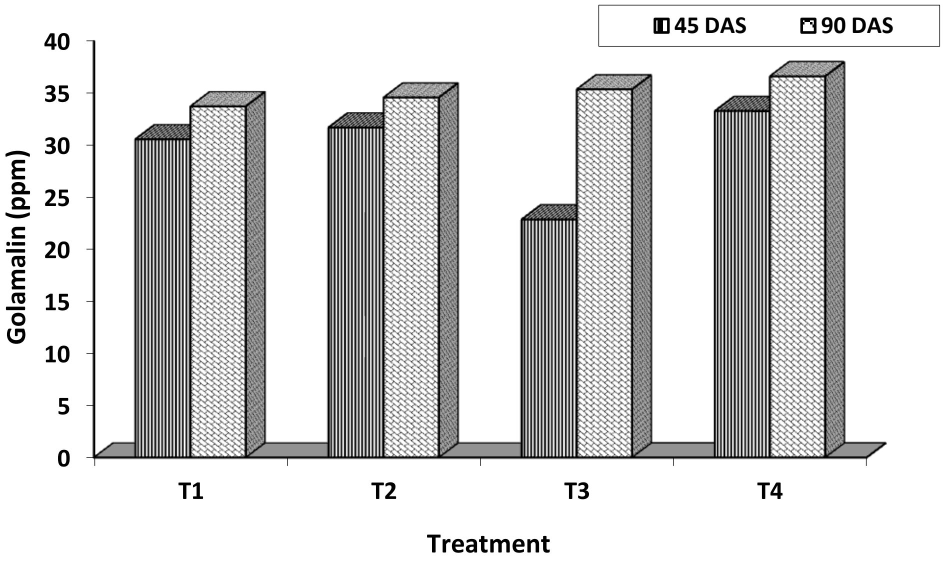
The effects of treatment had significant effect on *glomalin* in the spinach crop which was 35.13 (mg kg^{-1}) (T3) and 33.16 (mg kg^{-1}) (T2) respectively (Table, Fig 34.1 Appendix 1.19). The T4 (RDF+AM+SSP) was found most prominent effect *glomalin* of (38.26 mg kg^{-1}) where as T1 (RDF) was found in the least *glomalin* (32.17 mg kg^{-1}) in the spinach crop. The stages of the crop growth had significant effect of *glomalin* on the spinach crop. In 90 DAS (36.74 mg kg^{-1}) the mustard was much the higher available *glomalin* than the 45 DAS (29.62 mg kg^{-1}). The interaction effect between growing stage and treatment was observed in non-significant.

Table 34.2 Effect of phytoremediation on glomalin (mg kg^{-1}) content in soil during spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	22.56	42.52	32.54
T2	**RDF+SSP**	34.18	44.32	39.25
T3	**RDF+AM**	36.54	45.23	40.88
T4	**RDF+SSP+AM**	41.11	55.69	48.40
Mean		33.60	46.94	
		SEm±	**CD at 5% level**	
Treatment (T)		3.51	10.53	**SIG**
Stage (S)		2.48	7.44	**SIG**
Interaction (TxS)		4.30	12.89	**NS**

The effects of treatment had significant effect on *glomalin* in the spinach crop which was 40.88 (mg kg^{-1}) (T3) and 39.25 (mg kg^{-1}) (T2) respectively (Table, Fig 34.2 and Appendix 1.19). The T4 (RDF+AM+SSP) was found most prominent effect

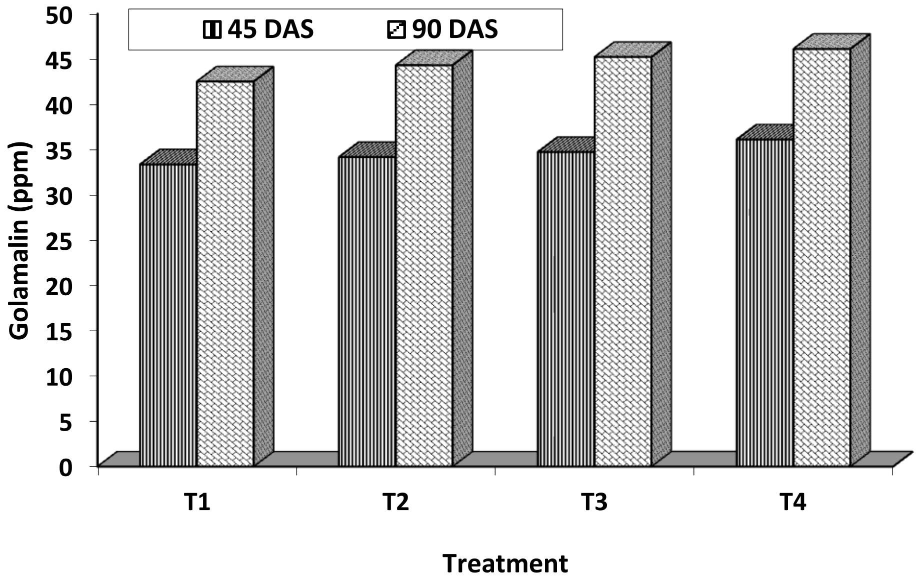
Fig 34.1 Effect of phytoremediation on golamalin (ppm) content in soil during spinach crop under cadmium spiked soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Fig 34.2 Effect of phytoremediation on golamalin (ppm) content in soil during spinach crop under natural cadmium contaminated soil



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

glomalin of (48.40 mg kg^{-1}) where as T1 (RDF) was found in the least *glomalin* (32.54 mg kg^{-1}) in the spinach crop. The stages of the crop growth had significant effect of *glomalin* on the spinach crop. In 90 DAS (46.94 mg kg^{-1}) the mustard had much the higher available *glomalin* than the 45 DAS (33.60 mg kg^{-1}). The interaction effect between growing stage and treatment was observed in non-significant.

Table 35.1 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during spinach crop under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	2.22	2.78	2.50
T2	**RDF+SSP**	2.78	3.56	3.17
T3	**RDF+AM**	3.00	3.89	3.44
T4	**RDF+SSP+AM**	3.44	5.22	4.33
Mean		2.86	3.89	
		SEm±	**CD at 5% level**	
Treatment (T)		0.276	0.827	**SIG**
Stage (S)		0.195	0.584	**SIG**
Interaction (TxS)		0.338	1.012	**NS**

The effects of treatment had significant effect on fungi in the spinach which was found 3.44 (cfu) (T3) and 3.17 (T2) respectively (Table, Fig 35.1 and Appendix 1.20). The T4 (RDF+AM+SSP) was found most prominent effect of available fungi 4.33 cfu where as T1 (RDF) was found in the least fungi 2.50 cfu in the spinach crop. The stages of the crop growth had significant effect of fungi on the spinach crop. In 45 DAS (2.86 cfu) the spinach was much the lower fungi than the 90 DAS (3.86 cfu). The interaction effect between growing stage and treatment was observed non-significant role in the available fungi in spinach crop.

Table 35.2 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	90 DAS	Mean
T1	**RDF**	2.33	3.11	2.72
T2	**RDF+SSP**	3.44	4.89	4.17
T3	**RDF+AM**	3.89	5.56	4.72
T4	**RDF+SSP+AM**	4.22	6.67	5.44
Mean		3.47	5.06	
		SEm±	**CD at 5% level**	
Treatment (T)		0.423	1.268	**SIG**
Stage (S)		0.299	0.896	**SIG**
Interaction (TxS)		0.518	1.553	**NS**

The effects of treatment had significant effect on fungi in the spinach crop which was observed 4.72 cfu (T3) and 4.17 cfu (T2) respectively (Table, Fig 35.2 and Appendix 1.21). The T4 (RDF+AM+SSP) was found most prominent effect fungi (5.44 cfu) where as T1 (RDF) was found in the least fungi (2.72 cfu) in the spinach crop. The stages of the crop growth had significant effect of fungi on the spinach crop. In 45 DAS (3.47 cfu) the spinach was much the minimum fungi than the 90 DAS (5.06 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

Table 36.1 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during spinach crop under cadmium spiked soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	90 DAS	Mean
T1	**RDF**	12.44	12.78	12.61
T2	**RDF+SSP**	12.67	13.00	13.33
T3	**RDF+AM**	14.33	14.22	14.78
T4	**RDF+SSP+AM**	16.11	16.11	16.11
Mean		13.89	14.53	
		SEm±	**CD at 5% level**	
Treatment (T)		0.205	0.613	**SIG**
Stage (S)		0.145	0.434	**SIG**
Interaction (TxS)		0.251	0.751	**NS**

The effects of treatment had significant effect on bacteria in the spinach which was found 14.78 (cfu) (T3) and 13.33 (cfu) (T2) respectively (Table, Fig 36.1 and Appendix 1.22). The T4 (RDF+AM+SSP) was observed most prominent effect of

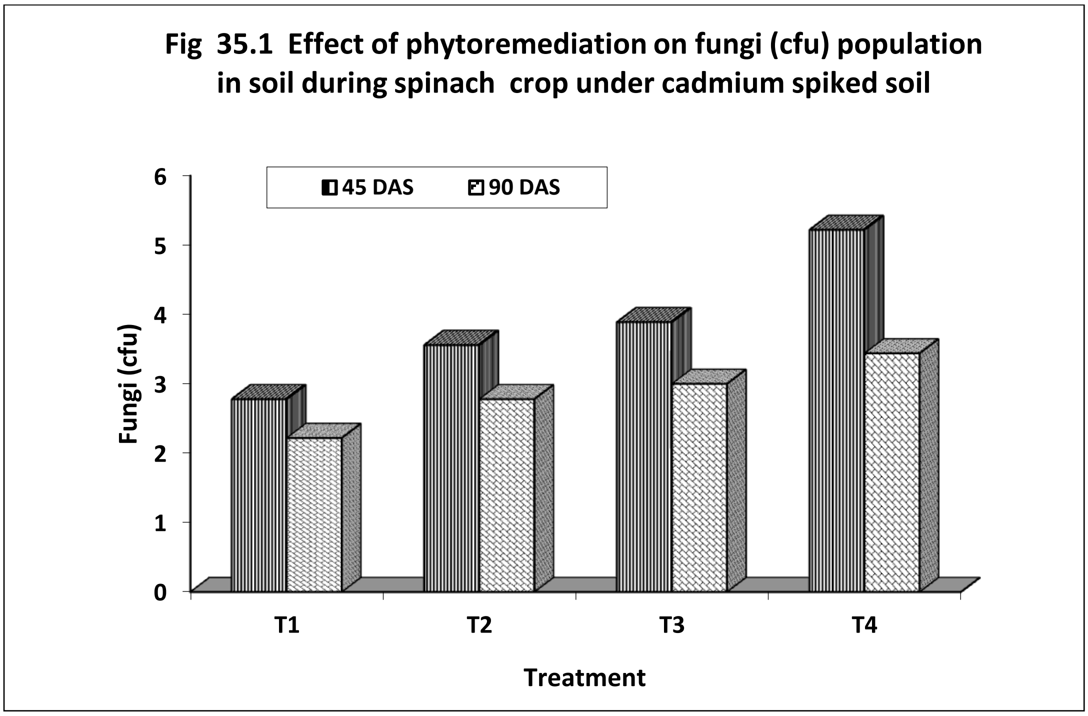


Fig 35.1 Effect of phytoremediation on fungi (cfu) population in soil during spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

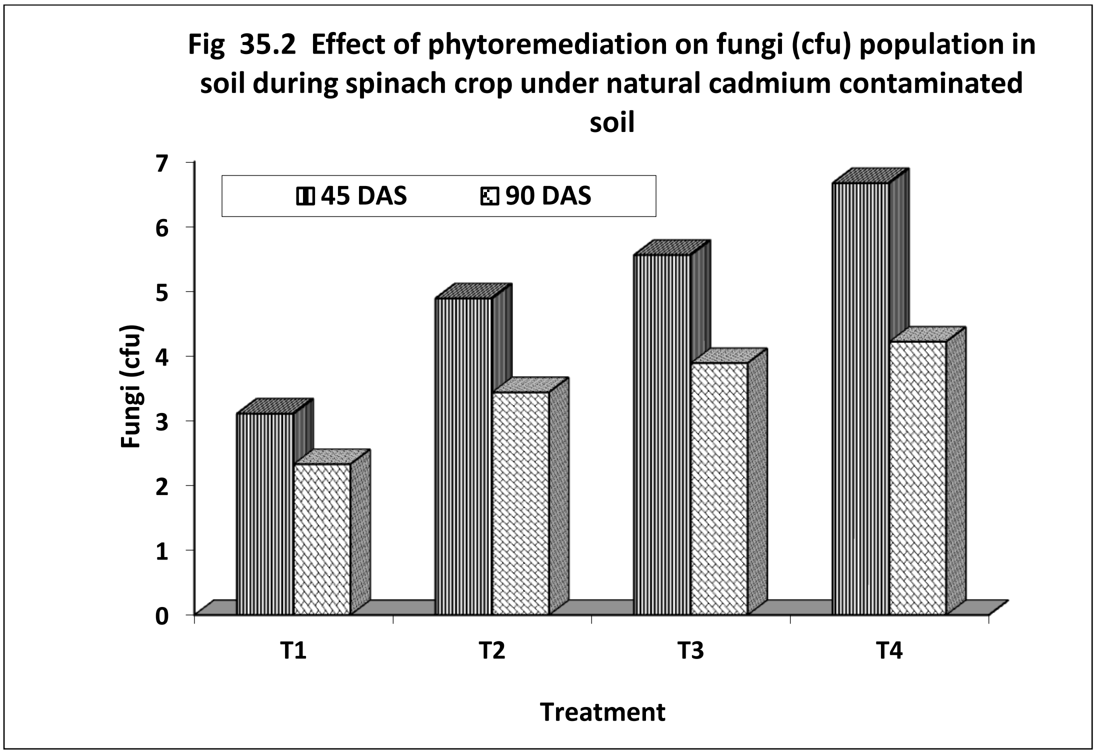


Fig 35.2 Effect of phytoremediation on fungi (cfu) population in soil during spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

bacteria 16.11 (cfu) where as T1 (RDF) was found in the least bacteria 12.61(cfu) in the spinach crop. The stages of the crop growth had significant effect of bacteria on the spinach crop. In 90 DAS (14.53 cfu) the spinach was much the higher bacteria than the 45 DAS (13.89 cfu). The interaction effect between growing stage and treatment had non-significant role in the available bacteria in spinach crop.

Table 36.2 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	14.67	18.22	16.44
T2	**RDF+SSP**	15.00	21.00	18.00
T3	**RDF+AM**	15.56	23.67	19.61
T4	**RDF+SSP+AM**	16.67	25.67	21.17
Mean		15.48	22.14	
		SEm±	**CD at 5% level**	
Treatment (T)		1.75	5.24	**NS**
Stage (S)		1.24	3.70	**SIG**
Interaction (TxS)		2.14	6.42	**NS**

The effects of treatment had non-significant effect on bacteria in the spinach crop (Table, Fig 36.2 and Appendix 1.21). The stages of the crop growth had significant effect of available bacteria on the spinach crop. In 90 DAS (15.48 cfu) the spinach was much the higher bacteria than the 45 DAS (22.14 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

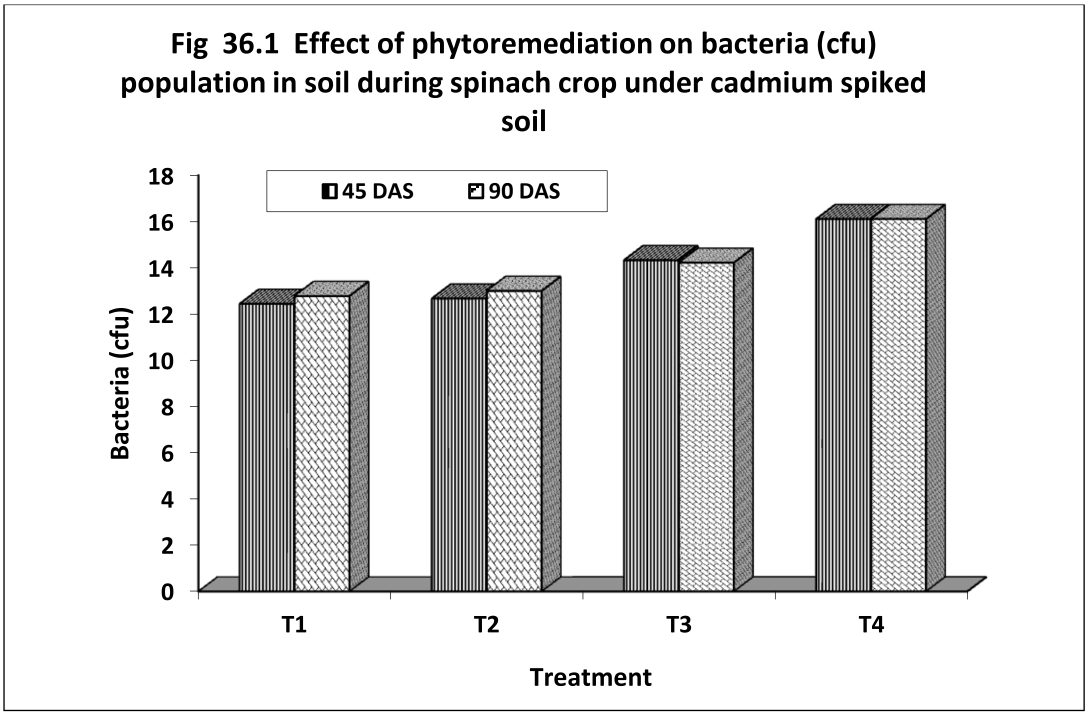


Fig 36.1 Effect of phytoremediation on bacteria (cfu) population in soil during spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

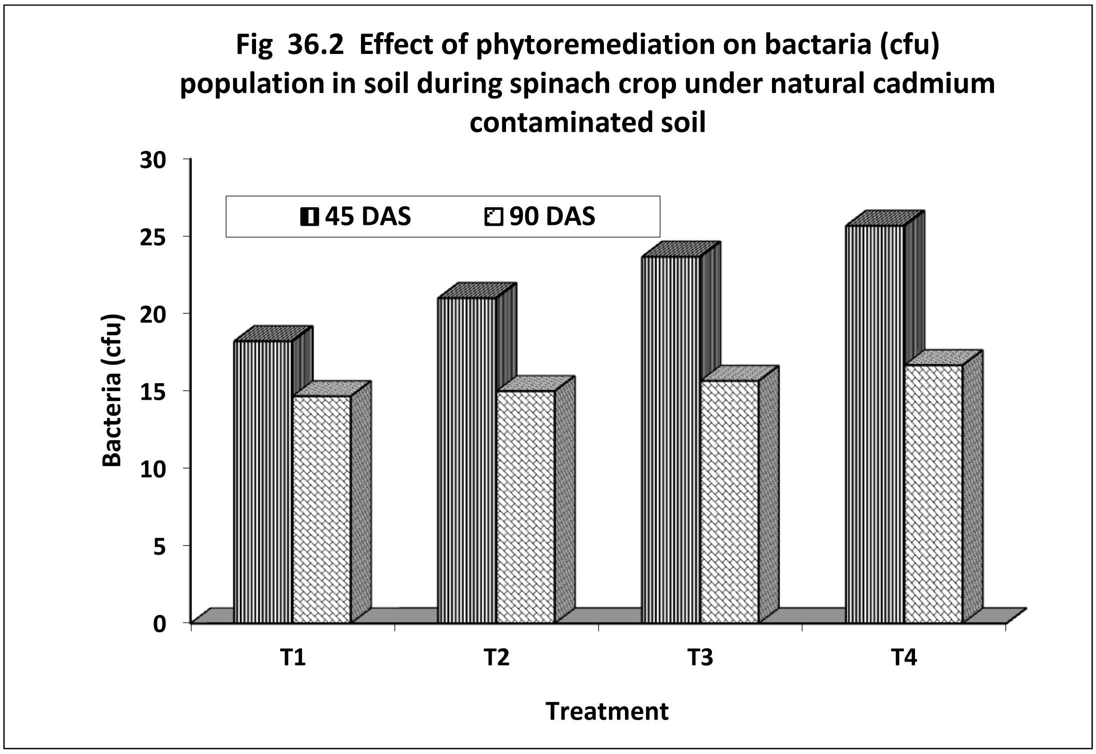


Fig 36.2 Effect of phytoremediation on bactaria (cfu) population in soil during spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 37.1 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during spinach crop under cadmium spiked soil

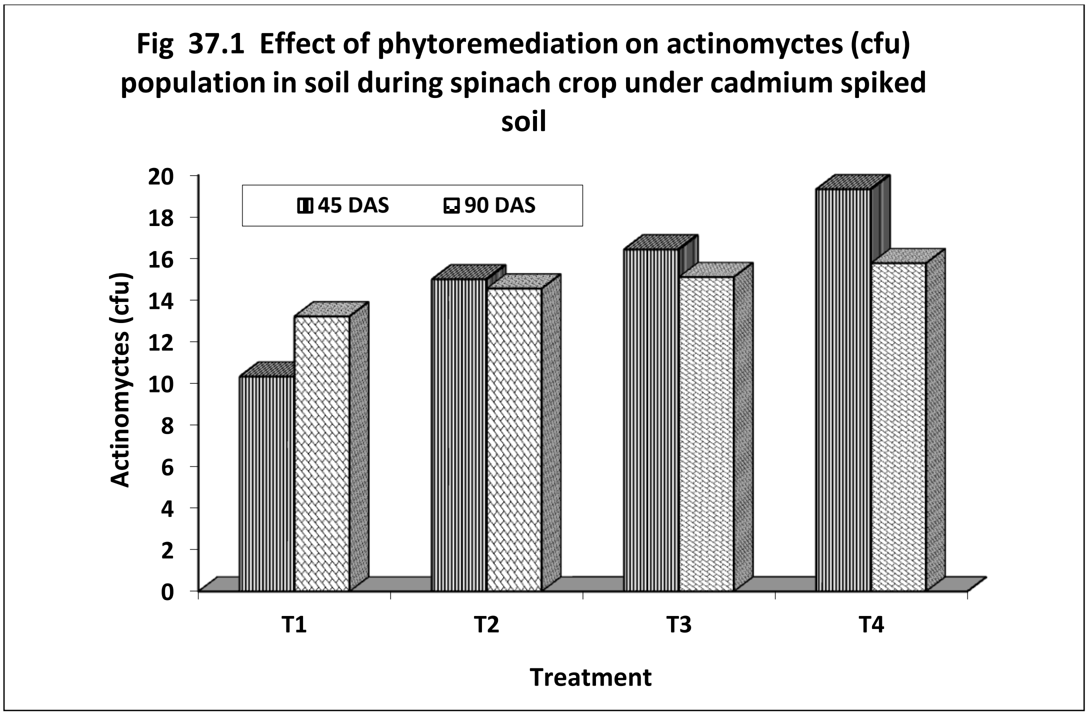
Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	13.22	10.33	11.78
T2	**RDF+SSP**	14.56	15.00	14.78
T3	**RDF+AM**	15.11	16.44	15.78
T4	**RDF+SSP+AM**	15.78	19.33	17.56
Mean		14.67	15.28	
		SEm±	**CD at 5% level**	
Treatment (T)		0.599	1.796	**SIG**
Stage (S)		0.424	1.270	**NS**
Interaction (TxS)		0.734	2.199	**SIG**

The effects of treatment had significant effect on available actinomycetes in the spinach which was found 15.78 (cfu) (T3) and 14.78 (cfu) (T2) respectively (Table, Fig 37.1 and Appendix 1.22). The T4 (RDF+AM+SSP) was found most prominent effect of actinomycetes 17.56 (cfu) where as T1 (RDF) was found in the least actinomycetes 11.78 (cfu) in the spinach crop. The stages of the crop growth had non-significant effect of actinomycetes on the spinach crop. The interaction effect between growing stage and treatment was observed significant role in the actinomycetes in spinach crop.

Table 37.2 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

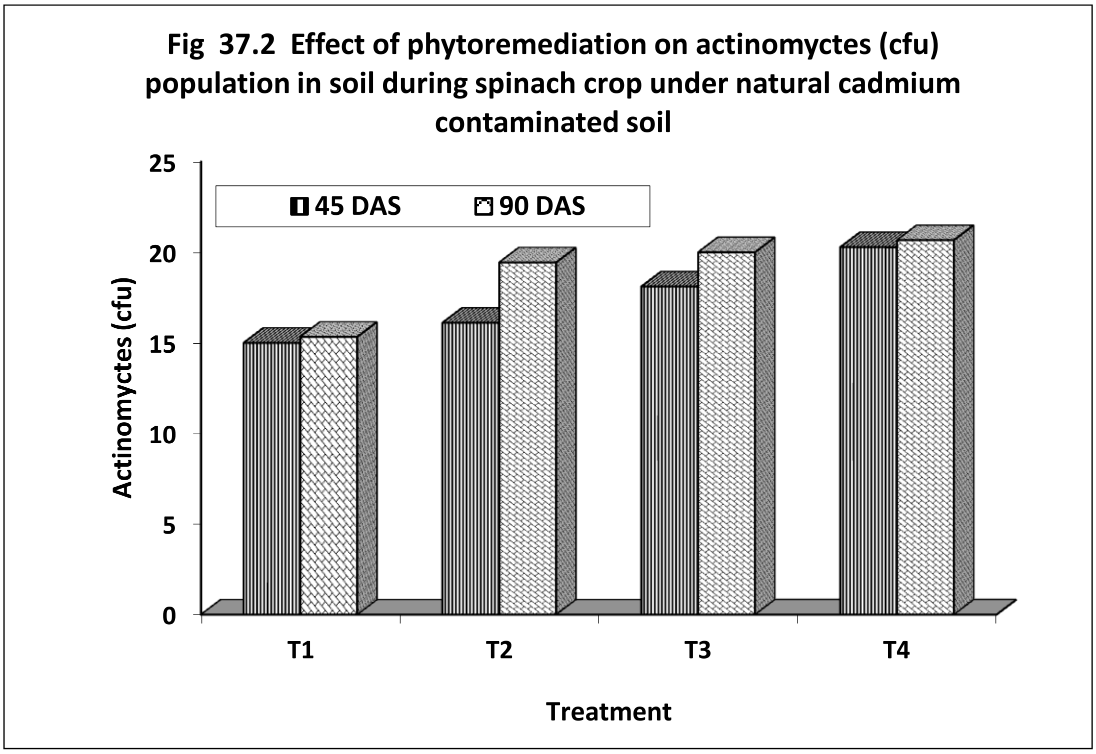
Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	15.00	15.33	15.17
T2	**RDF+SSP**	16.11	19.44	17.78
T3	**RDF+AM**	18.11	20.00	19.06
T4	**RDF+SSP+AM**	20.28	20.67	20.72
Mean		17.38	18.86	
		SEm±	**CD at 5% level**	
Treatment (T)		0.481	1.442	**SIG**
Stage (S)		0.340	1.020	**SIG**
Interaction (TxS)		0.589	1.766	**NS**

The effects of treatment had significant effect on actinomycetes in the spinach crop which was observed 19.06 (cfu) (T3) and 17.78 (cfu) (T2) respectively (Table, Fig 37.2 and Appendix 1.22). The T4 (RDF+AM+SSP) was found most prominent



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

effect actinomycetes (20.72 cfu) where as T1 (RDF) was found in the least actinomycetes no. (15.17 cfu) in the spinach crop. The stages of the crop growth had significant effect of actinomycetes on the spinach crop. In 90 DAS (18.86 cfu) the spinach was much the higher actinomycetes than the 45 DAS (17.38 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

Table 38.1 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during spinach crop under cadmium spiked soil

Treatment		**Stage of plant growth (spinach)**		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	19.00	19.67	19.33
T2	**RDF+SSP**	20.33	27.11	23.72
T3	**RDF+AM**	23.00	30.89	26.94
T4	**RDF+SSP+AM**	25.67	33.22	29.44
Mean		22.00	27.72	
		SEm±	**CD at 5% level**	
Treatment (T)		1.61	4.82	**SIG**
Stage (S)		1.14	3.41	**SIG**
Interaction (TxS)		1.97	5.91	**NS**

The effects of treatment had significant effect on N-fixer in the spinach which was found no. of 26.94 (cfu) (T3) and 23.72 (cfu) (T2) respectively (Table, Fig 38.1 Appendix 1.21). The T4 (RDF+AM+SSP) was found most prominent effect of N-fixer 29.44 (cfu) where as T1 (RDF) was found in the least N-fixer 19.33 (cfu) in the spinach crop. The stages of the crop growth had significant effect of N-fixer on the spinach crop. In 45 DAS (25.67 cfu) the spinach had much the lower N-fixer than the 90 DAS (31.94 cfu). The interaction effect between growing stage and treatment was observed non-significant role in the N-fixer in spinach crop.

Table 38.2 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

Treatment		Stage of plant growth (spinach)		
		45 DAS	**90 DAS**	**Mean**
T1	**RDF**	21.22	28.67	24.94
T2	**RDF+SSP**	24.89	30.44	27.67
T3	**RDF+AM**	26.67	33.33	30.00
T4	**RDF+SSP+AM**	29.89	35.33	32.61
Mean		25.67	31.94	
		SEm±	**CD at 5% level**	
Treatment (T)		1.58	4.75	**SIG**
Stage (S)		1.12	3.36	**SIG**
Interaction (TxS)		1.94	5.81	**NS**

The effects of treatment had significant effect on N-fixer in the spinach crop which was observed 30.00 (cfu) (T3) and 27.67 (cfu) (T2) respectively (Table, Fig 38.2 Appendix 1.22). The T4 (RDF+AM+SSP) was found most prominent effect N-fixer (32.61 cfu) where as T1 (RDF) was found in the least available N-fixer (24.94 cfu) in the spinach crop. The stages of the crop growth had significant effect of N-fixer on the spinach crop. In 90 DAS (25.67 cfu) the spinach was much the lower N-fixer than the 45 DAS (31.94 cfu). The interaction effect between growing stage and treatment was observed in non-significant.

Table 39.1 Effect of phytoremediation by mustard on cadmium removal (%) in cadmium spiked soil

Treatment		Stage of plant growth (mustard)		
		45 Days	**90 Days**	**Mean**
T1	**RDF**	47.83	56.55	52.19
T2	**RDF+SSP**	38.64	43.20	40.92
T3	**RDF+AM**	27.85	32.90	30.38
T4	**RDF+SSP+AM**	43.71	52.44	48.07
Mean		39.51	46.27	
		SEm	**CD at % level**	
Treatment (T)		1.76	5.28	**SIG**
Stage (S)		1.25	3.73	**SIG**
Interaction (TxS)		2.16	6.47	**NS**

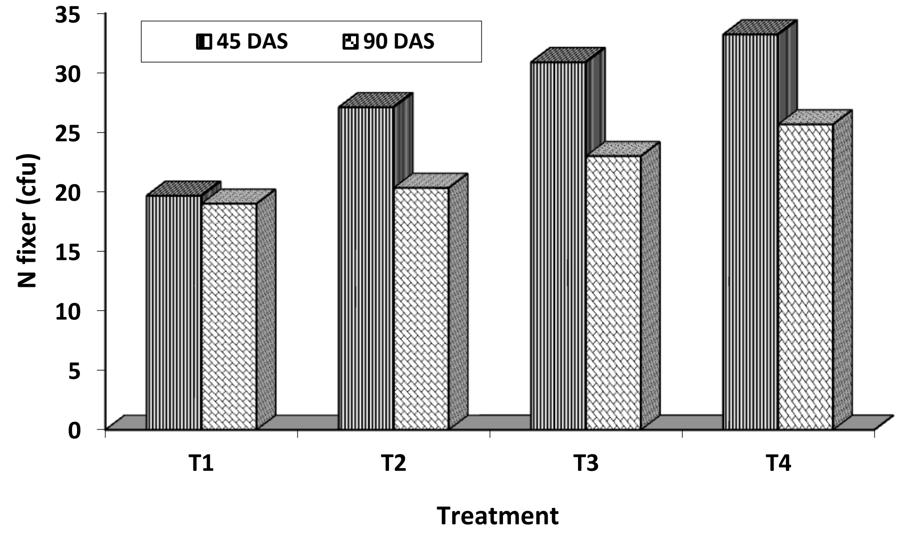


Fig 38.1 Effect of phytoremediation on N fixer (cfu) population in soil during spinach crop under cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

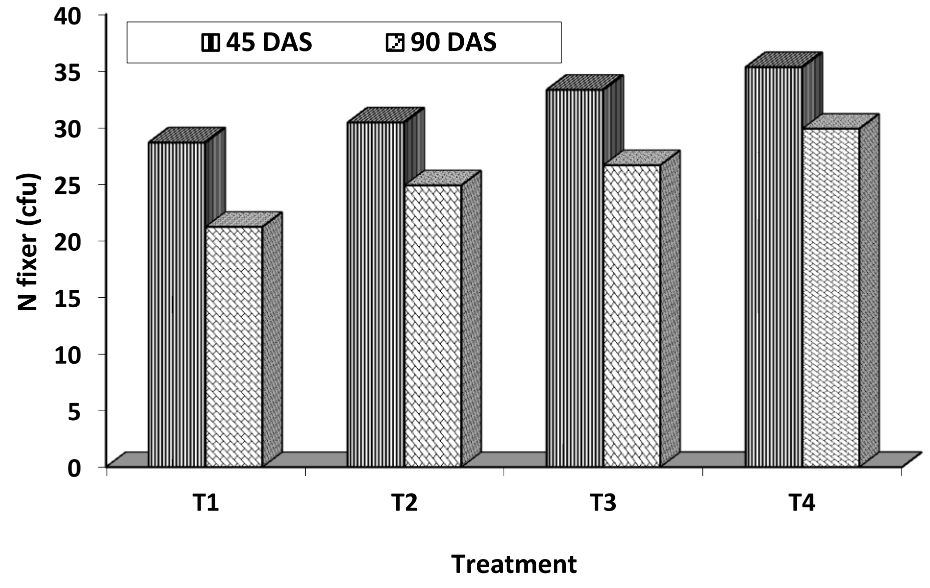


Fig 38.2 Effect of phytoremediation on N fixer (cfu) population in soil during spinach crop under natural cadmium contaminated soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

The effects of treatment had significant effect on removal Cd in the mustard which was 48.07 % (T4) and 40.92 % (T2) respectively (Table, Fig 39.1 and Appendix 1.23). The T1 (RDF) was found most prominent effect of removal Cd 52.19 % where as T3 (RDF+AM) was found in the least Cd removal 30.38 % in the mustard crop. The stages of the crop growth had significant effect of Cd removal on the mustard crop. In 45 DAS (39.51 %) the mustard had much the lower N-fixer than the 90 DAS (46.27 %). The interaction effect between growing stage and treatment was showed non-significant role in the Cd removal in mustard crop.

Table 39.2 Effect of phytoremediation by mustard on cadmium removal (%) in naturally cadmium contaminated soil

Treatment		**Stage of plant growth (mustard)**		
		45 Days	**90 Days**	**Mean**
T1	**RDF**	78.95	83.59	81.27
T2	**RDF+SSP**	74.48	81.11	77.79
T3	**RDF+AM**	70.34	79.12	74.73
T4	**RDF+SSP+AM**	77.13	82.10	79.62
Mean		75.23	81.48	
		SEm	**CD at % level**	
Treatment (T)		1.62	4.85	**NS**
Stage (S)		1.14	3.43	**SIG**
Interaction (TxS)		1.98	5.94	**NS**

The effects of treatment had non-significant effect on Cd removal in the mustard crop (Table, Fig 39.2 and Appendix 1.23). The stages of the crop growth had significant effect of Cd removal on the mustard crop. In 45 DAS (75.23 %) the mustard was much the lower Cd removal than the 90 DAS (81.48 %). The interaction effect between growing stage and treatment was observed in non-significant.

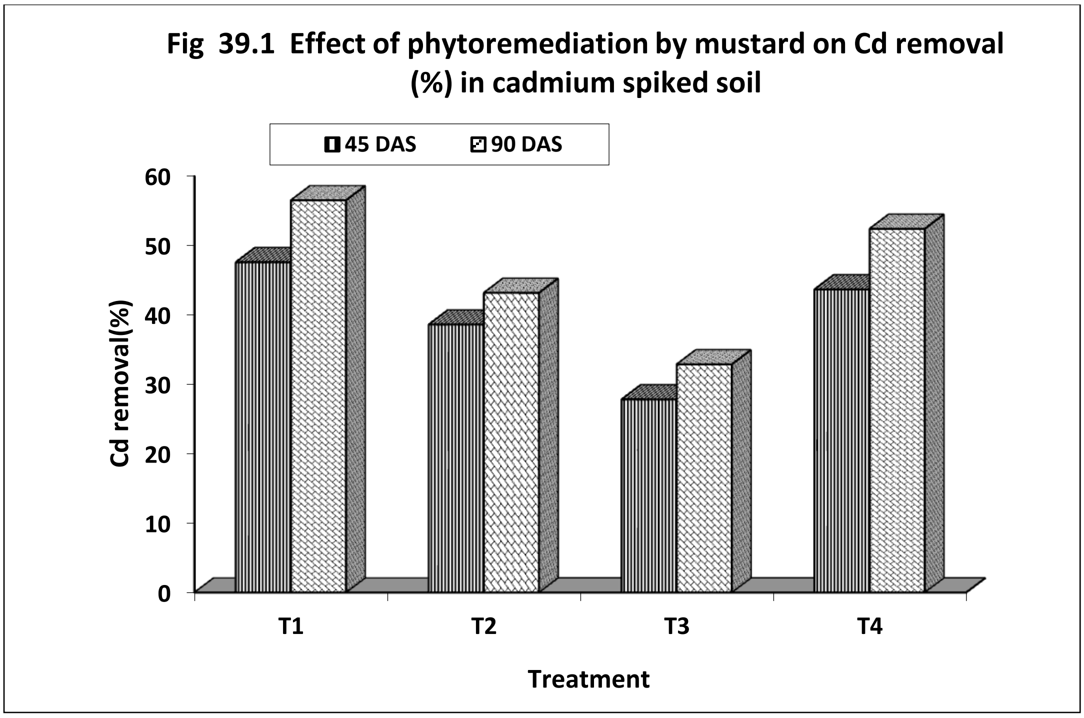


Fig 39.1 Effect of phytoremediation by mustard on Cd removal (%) in cadmium spiked soil

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

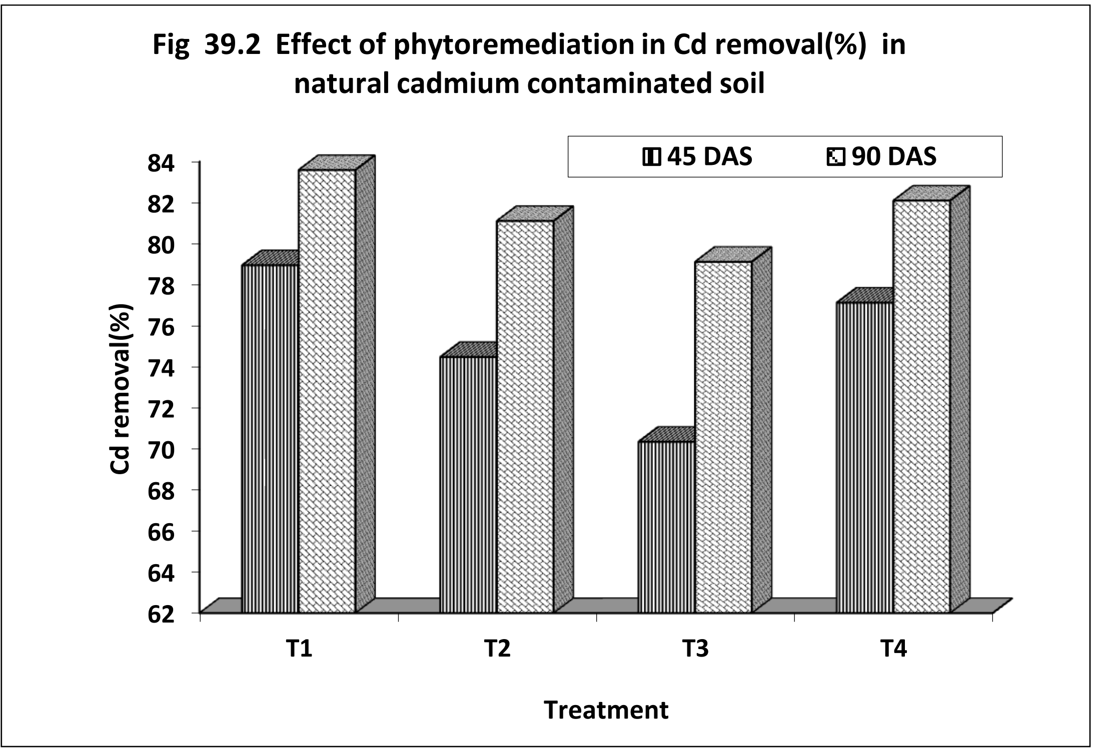


Fig 39.2 Effect of phytoremediation in Cd removal(%) in natural cadmium contaminated soil

1

Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

Table 40.1 Effect of phytoremediation by spinach on cadmium removal (%) in cadmium spiked soil

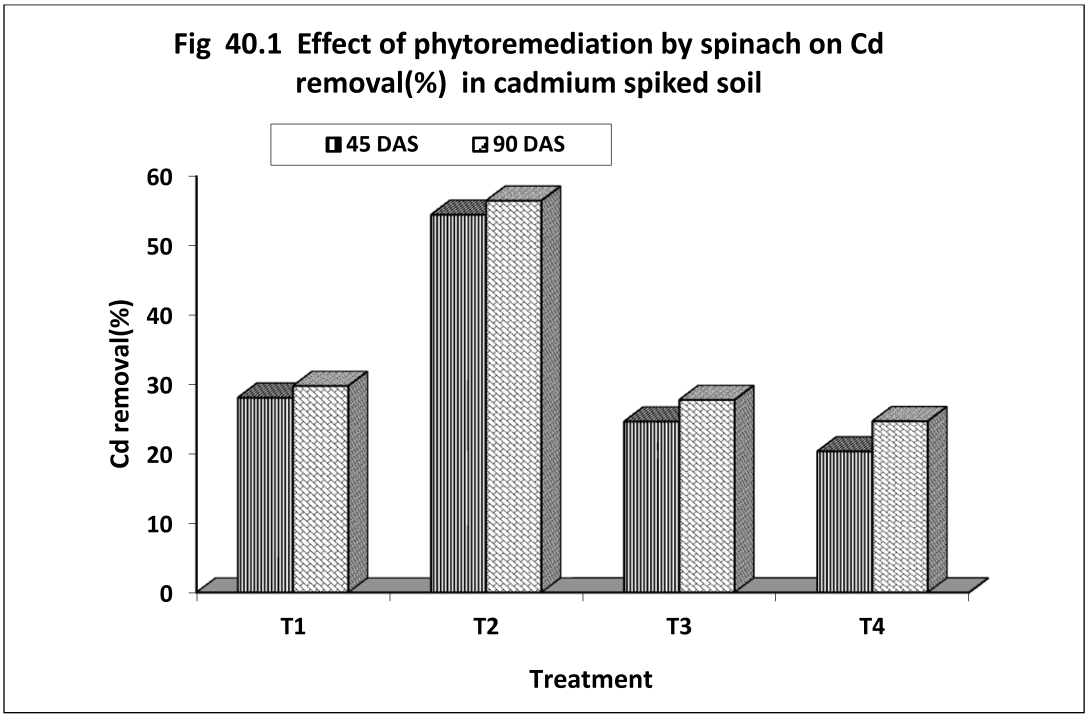
Treatment		Stage of plant growth (mustard)		
		45 Days	90 Days	Mean
T1	RDF	28.09	29.78	28.93
T2	RDF+SSP	54.46	56.49	55.48
T3	RDF+AM	24.65	27.77	26.21
T4	RDF+SSP+AM	20.34	24.70	22.52
Mean		31.88	34.69	
		SEm	CD at % level	
Treatment (T)		0.75	2.24	SIG
Stage (S)		0.53	1.58	SIG
Interaction (TxS)		0.92	2.75	NS

The effect of treatment had significant effect on removal Cd in the spinach which was 28.93 % (T1) and 26.21 % (T3) respectively (Table, Fig 40.1 and Appendix 1.23). The T2 (RDF+SSP) was found most prominent effect of removal Cd 55.45 % where as T4 (RDF+SSP+AM) was found in the least removal Cd 22.52 % in the spinach crop. The stages of the crop growth had significant effect of removal Cd on the spinach crop. In 45 DAS (31.88 %) the spinach was much the lower removal Cd than the 90 DAS (34.69 %). The interaction effect between growing stage and treatment was observed non-significant role in the Cd removal in spinach crop.

Table 40.2 Effect of phytoremediation by spinach on cadmium removal (%) in naturally cadmium contaminated soil

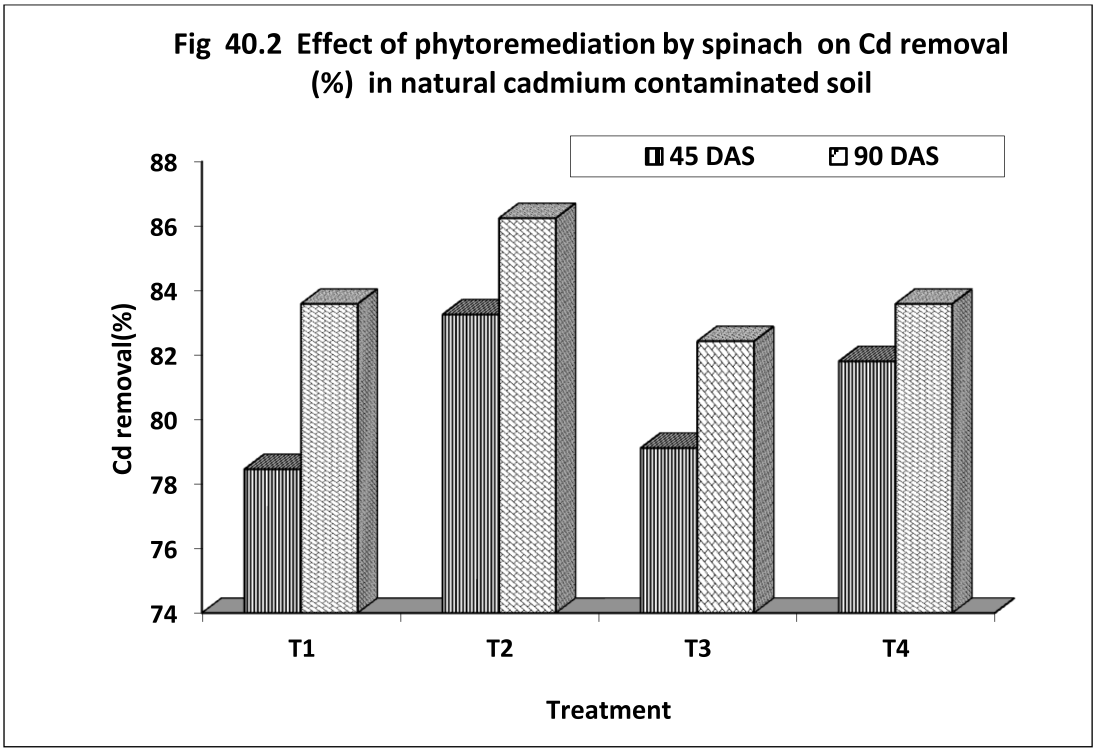
Treatment		Stage of plant growth (mustard)		
		45 Days	90 Days	Mean
T1	RDF	78.46	83.59	81.03
T2	RDF+SSP	83.26	86.25	84.75
T3	RDF+AM	79.12	82.43	80.78
T4	RDF+SSP+AM	81.61	83.59	82.60
Mean		80.61	83.97	
		SEm	CD at % level	
Treatment (T)		0.89	2.66	SIG
Stage (S)		0.63	1.88	SIG
Interaction (TxS)		1.08	3.25	NS

The effects of treatment had significant effect on removal Cd in the spinach crop which was observed 82.60 % (T4) and 81.03 % (T1) respectively (Table, Fig 40.2 and Appendix 1.23). The T2 (RDF+SSP) was found most prominent effect



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM



Treatment

T1 = Recommended dose of Fertilizer (RDF), **T2** = RDF + Single super phosphate (SSP), **T3** = RDF + Vesicular arbuscular mycorrhiza (AM), **T4** = RDF +SSP + AM

removal Cd (84.75 %) where as T3 (RDF+AM) was found in the least removal Cd (80.78 %) in the spinach crop. The stages of the crop growth had significant effect of removal Cd on the spinach crop. In 45 DAS (80.61 %) the spinach was much the lower removal Cd than the 90 DAS (83.97%). The interaction effect between growing stage and treatment was observed in non-significant.

CHAPTER – IV

DISCUSSION

1.1 Effect of phytoremediation on N content (%) in mustard shoot under cadmium spiked soil

Mycorrhizal infection may affect the mineral nutrition of the host plant directly by enhancing plant growth through nitrogen acquisition by the fungus, or indirectly by modifying transpiration rates and the composition of rhizosphere microflora (Marschner and Dell, 1994). The rate of N uptake of crops is highly AMriable during crop development. Critical N concentration i.e. the nitrogen concentration required for the maximum growth rate declines during crop growth (Gastal and Lemaire, 2002). Similar observation was observed in this study that with the advancement of growth period the N concentration in the plant declines.

1.2 Effect of phytoremediation on N content (%) in mustard shoot under naturally cadmium contaminated soil

Similar to cadmium spiked soil, in the naturally Cd contaminated soil the effect of phytoremediation with respect to stages of the crop growth have beneficial role in improving the N content in mustard shoot.

1.3 Effect of phytoremediation on N content (%) in mustard root under cadmium spiked soil

The agricultural practices and mycorrhizal inoculation increase the concentration of mineral nutrition in the soil solution and enhance the capacity to uptake by their root cells (Lynch, 2011). Increased vesicular arbuscular mycorrhizal infection increased the rate of N uptake by the plants (Ames *et al.,* 1984).

1.4 Effect of phytoremediation on N content (%) in mustard root under naturally cadmium contaminated soil

Similar to cadmium spiked soil the application vesicular arbuscular mycorrhizae (AM) and phosphates (SSP) along with recommended doses of fertilizer have improved the N content in the mustard root. The mycorrhiza and phosphates may influence on the N bioavailability in the soil which in turn influences on N content in the mustard root.

1.5 Effect of phytoremediation on N content (%) in mustard grain under cadmium spiked soil

Here it is proved that the effect of phytoremediation with respect to the stages of crop growth have great influence on the N content in mustard grain.

1.6 Effect of phytoremediation on N content (%) in mustard grain under naturally cadmium contaminated soil

Similar to Cd spiked soil, the stages of the crop growth has significant impact on the nitrogen accumulation in the in mustard grain under naturally Cd contaminated soil.

2.1 Effect of phytoremediation on P content (%) in mustard shoot under cadmium spiked soil

AM fungi play critical role in mobilizing P through the extensive growth of hyphae in the soil systems. In mycorrhizal association the fungal hyphae play an important role in the acquisition of P for the plant (Bolan, 1991; Smith and Read, 1997). Shen *et al.,* 2006 studied about the application of mycorrhizal plants and its potentiality to land decontamination with increased biomass and plant phosphorus nutrition by changing metal transfer. Mychorhizal species influences metal toxicity to plants through decreasing translocation of heavy metals and its concentration.

2.2 Effect of phytoremediation on P content (%) in mustard shoot under naturally cadmium contaminated soil

In contrast to Cd spiked soil, the stages of crop growth was found significant effect on P accumulation whereas the treatment effects was observed non significant, it may be due to under Cd stress condition only the AM and phopsphates application have prominent effect on P content in mustard shoot.

2.3 Effect of phytoremediation on P content (%) in mustard root under cadmium spiked soil

The stage of the crop has significant impact on improving P content in mustard root under Cd spiked soil. Physiological stages of the crop growth have major effect on the root N content in mustard.

2.4 Effect of phytoremediation on P content (%) in mustard root under naturally cadmium contaminated soil

Similar to Cd spiked soil, the P accumulation in mustard root is significantly affected by the stages of the crop growth. The less content of P was found in 90 DAS may be due maximum translocation from root to shoot took place by this period.

2.5 Effect of phytoremediation on P content (%) in mustard grain under cadmium spiked soil

The stage of the crop growth with respect to phytoremediation has significant impact in influencing the P content in the mustard crop under cadmium spiked soil.

2.6 Effect of phytoremediation on P content (%) in mustard grain under naturally cadmium contaminated soil

Within 75 DAS the maximum grain P accumulation took place than the 90 DAS. Similar to Cd spiked soil the stage of the crop growth has significant influence in P content in the mustard grain.

3.1 Effect of phytoremediation on K content (%) in mustard shoot under cadmium spiked soil

This study revealed that the stage of crop growth with respect to phytoremediation has larger impact on potassium content in the mustard shoot. Ciecko *et al.,* 2004 reported that the total content of potassium in the plants and the trend of its changes as the result of soil contamination with cadmium were affected mainly by plant species. They also confirmed that soil contamination with cadmium caused an increase or a decrease in the content of potassium, depending on the plant species and organ. An increase in the content of potassium was found in oat straw and roots and in the roots of maize and a decrease in the content of this element was observed in oat grains as well as in the above-ground parts and the roots of yellow lupine and radish.

3.2 Effect of phytoremediation on K content (%) in mustard shoot under naturally cadmium contaminated soil

In this study, like N and P the K content in the plant also in the plant decrease with the advancement of growing period in relation to phytoremediation. Similar to cadmium spiked soil, in the naturally Cd contaminated soil also have same impact on potassium content in the mustard shoot.

3.3 Effect of phytoremediation on K content (%) in mustard root under cadmium spiked soil

Similar to mustard shoot, the stages of crop growth with respect to phytoremediation has significant influence on the root K content in the mustard under Cd spiked soil.

3.4 Effect of phytoremediation on K content (%) in mustard plant root under naturally cadmium contaminated soil

Likewise in Cd spiked soil, the root content of K was found significantly higher in the earlier stages of crop growth than the later stages of the crop under naturally

Cd contaminated soil. The K content of the mustard root decreases with the advancement of the growing period.

3.5 Effect of phytoremediation on K content (%) in mustard grain under cadmium spiked soil

Similar to mustard shoot and root the K content in the mustard root is also significantly influenced by the stage of the crop growth with respect to phytoremediation. Here also the grain K content found higher during initial stage of crop growth than the latter stage of the growing period. The arbuscular mycorrhizal inoculation was may play critical role in improving the nutrient availability and meeting the nutrient deficiency in crops. Mycorrhizal colonization in plant root improves potassium uptake along with some major and micronutrients (Halder *et al.,* 2015).

3.6 Effect of phytoremediation on K content (%) in mustard grain under naturally cadmium contaminated soil

Similar to Cd spiked soil, in naturally contaminated soil also same trend in terms of K content in the mustard grain. Here also the stage of the crop growth has significant impact on the K content in the mustard grain.

4.1 Effect of phytoremediation on S content (%) in mustard shoot under cadmium spiked soil

The application of phosphatic fertilizer as well as mycorrhizal inoculation has significant impact in improving the S content in the mustard shoot. Mycorrhizal development in the rhizosphere of the mustard may have key role on the S availability and uptake by the crop.

4.2 Effect of phytoremediation on S content (%) in mustard shoot under naturally cadmium contaminated soil

Similar to Cd spiked soil, in naturally contaminated soil the application of phosphates (SSP) and AM mycorrhiza improved the S content in the mustard shoot. The mycorrhizal development in the rhizosphere is the main contributor to improve S content in the crop.

4.3 Effect of phytoremediation on S content (%) in mustard root under cadmium spiked soil

In case of naturally contaminated soil the S content is in mustard root is different that only the stage of the crop growth significantly influences the S content of the root.

4.4 Effect of phytoremediation on S content (%) in mustard root under naturally cadmium contaminated soil

In contrast to Cd spiked, the S content in the mustard root is significantly influenced by the treatments i.e. AM mycorrhiza and SSP application. Under little stress of Cd in naturally contaminated soil the S content in roots of mustard may improved.

4.5 Effect of phytoremediation on S content (%) in mustard grain under cadmium spiked soil

In this study it was proved that the application of AM mycorrhiza and phosphates (SSP) has significant contribution to increase the S accumulation in the mustard grain under cadmium spiked soil.

4.6 Effect of phytoremediation on S content (%) in mustard grain under naturally cadmium contaminated soil

Similar to Cd spiked soil, the S content in the mustard grain significantly influenced by the treatments and stages of the crop growth under naturally contaminated soil . Mycorrhiza and phosphates application has great role for the enhancement of S content in the mustard grain.

5.1 Effect of phytoremediation on Cd content (ppm) in mustard shoot under cadmium spiked soil

With the advancement of the growing period the Cd content in the shoot increases it may be due to its higher capability to accumulate and store in the shoots of the crop.

5.2 Effect of phytoremediation on Cd content (ppm) in mustard shoot under naturally cadmium contaminated soil

Like Cd spiked soil, the Cd concentration in the shoots increases with the advancement of the growing period. It was reported earlier that Indian mustard (*B. juncea*) was a better hyperaccumulator for Pb, Zn, and Cd (Indoria and Poonia, 2006; Rio *et al.,* 2000, 2004) than the other *Brassica spp.*

5.3 Effect of phytoremediation on Cd content (ppm) in mustard root under cadmium spiked soil

The Cd accumulation in mustard root is only affected by the stages of the crop growth. The latter stage of the plant growth has greater Cd accumulation which in turn may affect the Cd accumulation by the above ground parts (shoot and seed) of the crop.

5.4 Effect of phytoremediation on Cd content (ppm) in mustard root under naturally cadmium contaminated soil

Similar to Cd spiked soil, the Cd content in the mustard root affected by the stage of the crop growth. Stage of crop plants with respect to phytoremediation has great influence for Cd content in the mustard root.

5.5 Effect of phytoremediation on Cd content (ppm) in mustard grain under cadmium spiked soil

In the cadmium spiked soil, the Cd accumulation in the mustard grain is quite high that is very serious issues for the consumption purpose.

5.6 Effect of phytoremediation on Cd content (ppm) in mustard grain under naturally cadmium contaminated soil

In naturally contaminated soil, the degree of Cd accumulation in the mustard grain is less and it was significantly influenced by the treatments and stages of the crop growth.

6.1 Effect of phytoremediation on soil available N (kg ha^{-1}) in mustard under cadmium spiked soil

The stages of the crop growth has great contributing factor influencing the soil available N. The soil nutrient (N) availability also depends on the N requirements by the plants.

6.2 Effect of phytoremediation on soil available N (kg ha^{-1}) in mustard under naturally cadmium contaminated soil

Similar to Cd spiked soil, the soil N availability significantly affected by the growing stages of the mustard under naturally contaminated soil.

7.1 Effect of phytoremediation on soil available P (kg ha^{-1}) in mustard under cadmium spiked soil

The soil available P content improved by the treatment as well as growing stages of the crop due to fact the mycorrhize caused great solubiliztion of immobile phosphorus present in the soil and increases the P availability to the crop.

7.2 Effect of phytoremediation on soil available P (kg ha^{-1}) in mustard crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, P availability is highly influenced by the treatment and stages of the crop growth under naturally contaminated soil. Soil available P is largely dictates by the solubilization by the rhizopheres microbes.

8.1 Effect of phytoremediation on soil available K (kg ha^{-1}) in mustard crop under cadmium spiked soil

It was observed that AM mycorrhiza application has key role improving the phosphorus nutrition that can moderate or improve the K availability in the soil. The contribution of this mutualistic symbiosis to the enhancement of plant K^{+} nutrition is not well understood and poorly studied so far (Garcia and Zimmermann, 2014). Both the treatments (AM mycorrhiza and SSP application) and stages of crop growth has significant influence the soil available K in mustard crop under Cd spiked soil.

8.2 Effect of phytoremediation on soil available K (kg ha^{-1}) in mustard crop under naturally cadmium contaminated soil

In this soil the trend of soil available K exactly similar to Cd spiked soil. The mutualistic symbiosis can play great role for the enhancement of the K in the soil.

9.1 Effect of phytoremediation on soil available S (mg kg^{-1}) in mustard crop under cadmium spiked soil

The S availability in the soil was highly influenced by the treatments with respect to AM mycorrhiza and phosphates application. In Cd spiked soil, the stages of the crop growth doesn't have any influence on the soil available S.

9.2 Effect of phytoremediation on soil available S (mg kg^{-1}) in mustard crop under naturally cadmium contaminated soil

The S availability in the soil was significantly influenced by the AM mycorrhiza and SSP application as well as the growing stages of the crop.

10.1 Effect of phytoremediation on soil Cd (ppm) in mustard crop under cadmium spiked soil

In this study it is proved that application of AM mycorrhiza and phosphatic fertilizer (SSP) significantly influence on the soil Cd. Logically higher phytoextraction of metals should result in a low metal concentration in the soil. In the latter stage of crop growth (90 DAS) revealed that lower concentration of soil Cd. However, the addition of P source as monobasic calcium phosphate was found to immobilize Pb and Cd, whereas in case of Zn was slightly mobilized (Theodoratos *et al.,* 2002).

10.2 Effect of phytoremediation on soil Cd (ppm) in mustard crop under naturally cadmium contaminated soil

The lower the Cd concentration in the soil at the time of harvest may be due to more bioavailable fraction in the soil was transferred to the plants. The interaction effect between growing stage and treatment was found non-significant.

11.1 Effect of phytoremediation on dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$) in mustard crop under cadmium spiked soil

With respect to growing stages in mustard crop the dehydrogenase activity in the soil improved. As the Cd concentration decreases over the growth period the dehydrogenase activity in the soil improved. In a study by Mandal *et al.,* (2014) reported that the successive growing cycle with Chinese brake fern has improved the soil microbiological properties in arsenic contaminated soil. The bio available forms of Cd in the soil or in the soil solution highly correlate to its toxicity parameters (Vig *et al.,* 2003).

11.2 Effect of phytoremediation on dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$) in mustard crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, here also the stage of the crop growth has great influence on the soil dehydrogenase activity.

12.1 Effect of phytoremediation on fluorescein di-acetate (*µg* fluorescein g^{-1}) in mustard crop under cadmium spiked soil

With respect to growing stages of the crop the fluorescein di-acetate activity significantly enhanced this may be due to declining the soil available fraction of Cd in the soil under the Cd spiked soil. Growth of the phytoremediating plant might have a beneficial effect on soil health in a very short time, actually enhancing the activity and functionality of the soil microbial communities (Epelde *et al.,* 2010)

12.2 Effect of phytoremediation on fluorescein di-acetate (*µg* fluorescein g^{-1}) activity in mustard crop under naturally Cd contaminated soil

In the naturally Cd contaminated soil, the fluorescein di-acetate activity significantly influenced by the growing stages of the crop as well as by the application of mycorrhiza and phosphates.

13.1 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in mustard crop under cadmium spiked soil

In Cd spiked soil, the soil respiration has improved significantly due to application of AM mycorrhiza and phosphates (SSP). The AM mycorrhiza present in the rhizosphere may improve the influence the microbial activity and functional diversity that may improve soil respiration.

13.2 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in mustard crop under naturally cadmium contaminated soil

Similar to Cd spiked soil exactly same observation found that treatments has great influence on the soil respiration that may be due to AM mycorrhiza on soil microbial activity which in turns improves the soil respiration in naturally Cd contaminated soil.

14.1 Effect of phytoremediation on Soil microbial biomass carbon (SMBC) in mustard crop under cadmium spiked soil

Under cadmium spiked soil the soil, the soil microbial biomass carbon (SMBC) significantly improved by the stages of crop production during the process of phytoremediation. As the Cd accumulation in the plant increase the microbial biomasss in the soil increase due to less toxic effect of Cd for microbial proliferation. Mandal *et al.,* (2014) reported that repeated phytoextraction by Chinese brake fern improves the microbial biomass carbon in the soil.

14.2 Effect of phytoremediation on Soil microbial biomass carbon (SMBC) in mustard under naturally cadmium contaminated soil

Similar to Cd spiked soil, the SMBC was improved due to stages of the crop growth. This observation supported by the study of Mandal *et al.,* (2014).

15.1 Effect of phytoremediation on soil glomalin (mg kg^{-1}) content in soil during mustard under cadmium spiked soil

In the Cd spiked soil, the glomalin content significantly improved due to stage of the crop growth. With the stages of the crop growth and the treatments due to AM mycorrhiza and SSP, there was a improvement of the microbial activity that may influence on the production of glomalin content in the soil.

15.2 Effect of phytoremediation on glomalin (mg kg^{-1}) content in soil during mustard crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the glomalin content in the soil improved due to growing stage of the crop as well as due to the treatment application i.e. AM mycorrhiza and phosphates (SSP). The AM mycorrhiza has significant contribution for the production of glomalin in the soil.

16.1 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during mustard under cadmium spiked soil

In Cd spiked soil, the population of fungi significantly improved due to application of AM mycorrhiza and phosphates. The effect of treatments has great influence influencing the microbial growth and population that improves the fungi population in the soil.

16.2 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the fungi population is following same trend with respect to its treatment and stages of the crop growth. The effect of treatments and growing stage of the crop has great influence influencing the microbial growth and population that improves the fungi population in the soil.

17.1 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during mustard crop under cadmium spiked soil

In Cd spiked soil, the population of bacteria significantly improved due to application of AM mycorrhiza and phosphates. The effect of treatments and the stages of the crop growth have great influence influencing the microbial growth and population that improves the bacterial population in the soil.

17.2 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the bacterial population is following same trend with respect to its treatment and stages of the crop growth.

18.1 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during mustard crop under cadmium spiked soil

In Cd spiked soil, the population of actinomycetes significantly improved due to application of AM mycorrhiza and phosphates. The effect of treatments has great influence influencing the microbial growth and population that improves the actinomycetes population in the soil.

18.2 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

In the naturally contaminated soil, the actinomycetes population was greatly influenced by the treatment and stages of the crop growth.

19.1 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during mustard crop under cadmium spiked soil

In Cd spiked soil, the population of N fixers significantly improved due to application of AM mycorrhiza and phosphates as well due to crop growing stage.

The effect of treatments has great influence influencing the microbial growth and population that improves the N fixers population in the soil.

19.2 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during mustard crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the N-fixers population following same trend that may be due to enhanced microbial activity and their population could influence more number of N-fixers in the soil

20.1 Effect of phytoremediation on N content (%) in spinach plant crop under cadmium spiked soil

In the Cd spiked soil, the N content in the spinach shoot significantly improves with respect to its growing stage and due to interaction effects of growing stage and treatment.

20.2 Effect of phytoremediation on N content (%) in spinach plant crop under naturally contaminated soil

Similar to Cd spiked soil, the Nitrogen content also affected by the growing stage of the crop in the natural Cd contaminated soil. AMrious factors responsible for N accumulation in the plants such as nutritional, environmental and physiological factors (Anjana *et al.,* 2006; Maynard *et al.,* 1976).

20.3 Effect of phytoremediation on N content (%) in spinach root under cadmium spiked soil

Application of mycorrhiza and phosphatic fertilizer significantly influence the N content in the spinach root. The mycorrhizal inoculation and phosphates application improves the root growth that may improves the N content in the root.

20.4 Effect of phytoremediation on N content (%) in spinach root under naturally cadmium contaminated soil

Similar to Cd contaminated soil, the N content in the root significantly improves with respect to its growing stage and due to interaction effects of growing stage and treatment. This study indicates the mycorrhizal inoculation and phosphates improves the N content in the root.

21.1 Effect of phytoremediation on P content (%) in spinach plant under cadmium spiked soil

In the Cd spiked soil, stages of crop growth and interaction effects due to growing stage and treatments has significant influence on P acquisition by the spinach.

21.2 Effect of phytoremediation on P content (%) in spinach plant under naturally cadmium contaminated soil

Similar to Cd contaminated soil, the P content in the spinach shoot significantly improves with respect to its growing stage and due to interaction effects of growing stage and treatment.

Stages of crop growth has significant role on N accumulation in the plants, the interaction between stages. With the advancement in growing period the plant N content decreased. The factors responsible for nitrate accumulation in plants are mainly nutritional, environmental, and physiological. Nitrogen content in the plants arises with the physiological ages of the plant growth (Anjana *et al.,* 2006; Maynard *et al.,* 1976).

21.3 Effect of phytoremediation on P content (%) in spinach root under cadmium spiked soil

In the Cd spiked soil, stages of crop growth and interaction effects due to growing stage and treatments has significant influence on P acquisition by the spinach.

21.4 Effect of phytoremediation on P content (%) in spinach root under naturally cadmium contaminated soil

In the Cd contaminated soil, treatment and interaction effect are similar to Cd spiked soil and the crop growth stage are significant role in P content in spinach root.

22.1 Effect of phytoremediation on K content (%) in spinach plant under cadmium spiked soil

Under the Cd spiked soil, the K content in the spinach shoot significantly improves with respect to its growing stage and due to interaction effects of growing stage and treatment.

22.2 Effect of phytoremediation on K content (%) in spinach plant under naturally cadmium contaminated soil

In naturally Cd Contaminated soil, the stages of crop growth has significant influence on the crop growth. The interaction effects due to crop growth stage and due to treatment has great impact on the plant K content. The physiological and environmental factor has great influence on the plant K content.

22.3 Effect of phytoremediation on K content (%) in spinach root under cadmium spiked soil

Under the Cd spiked soil, the K content in the spinach root significantly improves with respect to its growing stage period of the K content in the crop decreases.

22.4 Effect of phytoremediation on K content (%) in spinach root under naturally cadmium contaminated soil

Similar to Cd spiked soil, the K content in the root significantly influence by the stages of the crop growth under the naturally Cd contaminated soil. With the advancement of the growing period the K content in the crop decreases that may be due to physiological stages of the crop growth.

23.1 Effect of phytoremediation on S content (%) in spinach plant under cadmium spiked soil

Under the Cd spiked soil, the S content in the spinach crop significantly influence by the stages of the crop and interaction effect due to growing stage and treatments.

23.2 Effect of phytoremediation on S content (%) in spinach plant under naturally cadmium contaminated soil

In naturally contaminated soil, only the interaction due to stages of the crop growth and treatments has play significant effect on the S content in the spinach crop.

23.3 Effect of phytoremediation on S content (%) in spinach root under cadmium spiked soil

In the Cd spiked soil, the S content in the roots is significantly influenced by the stages of the crop growth. It may be due to physiological stages crop growth and phytoremediation effect play a great role influencing the S content in the spinach root.

23.4 Effect of phytoremediation on S content (%) in spinach root under naturally cadmium contaminated soil

Similar to Cd contaminated soil, the S content in the spinach root significantly influence by the stages of the crop growth under naturally Cd contaminated soil.

24.1 Effect of phytoremediation on Cd content (ppm) in spinach plant under cadmium spiked soil

In the Cd spiked soil, the stages of the crop growth significantly influence on Cd accumulation in the spinach crop. With the advancement of the growing period the Cd accumulation trend is increasing. It is proved that spinach plant has great capacity to accumulate Cd. The interaction effect due to stages of the crop and treatment has significant role on influencing the Cd content on the spinach plant.

24.2 Effect of phytoremediation on Cd content (ppm) in spinach plant under naturally cadmium contaminated soil

Similar to Cd spiked soil, on Cd accumulation in the spinach crop greatly influence by the stages of the crop growth. With the advancement of the growing period the Cd accumulation trend is increasing. It is proved that spinach plant has great capacity to accumulate Cd. The interaction effect due to stages of the crop and treatment has significant role on influencing the Cd content on the spinach plant.

24.3 Effect of phytoremediation on Cd content (ppm) in spinach root under cadmium spiked soil

In the Cd spiked soil, the spinach root has also great capacity to accumulate Cd. Here the stage of crop has significant role in improving the Cd content in the root, with the advancement of the growing period the Cd accumulation in the crop was improved.

24.4 Effect of phytoremediation on Cd content (ppm) in spinach root under naturally cadmium contaminated soil

Like Cd spiked soil, the Cd content in the spinach root improved, as this soil initial less Cd contaminated soil, hence the degree of accumulation was little. With the advancement of the growing period of spinach plant, here also Cd accumulation was improved.

25.1 Effect of phytoremediation on soil available N (kg ha^{-1}) in spinach crop under cadmium spiked soil

In the Cd spiked soil, the soil available N was significantly improved due to treatments by means of application of AM mycorrhiza and phosphate application. The AM mycorrhiza play great role in the improvement of soil N content. With the advancement of growing period of the plants has the soil available N was improved.

25.2 Effect of phytoremediation on soil available N (kg ha^{-1}) in spinach crop under naturally cadmium contaminated soil

In the naturally Cd contaminated soil, the effect of treatments due to application of mycorrhiza and phosphates significantly influenced the soil available N content. The stages of the crop growth also have significance influence on the soil available N, this may be due to more availability leads to better uptake by the crop.

26.1 Effect of phytoremediation on soil available P (kg ha^{-1}) in spinach crop under cadmium spiked soil

In the Cd spiked soil, the effect of treatments due to application of mycorrhiza and phosphates has significant influence the soil available P content. AM Mycorrhiza play important role in mobilizing P in the soil (Rai *et al.,*, 2013). The stages of the crop growth also have significance influence on the soil available P, this may be due to more availability leads to better uptake by the crop. With the advancement of the crop growing period the P availability improved in the soil.

26.2 Effect of phytoremediation on soil available P (kg ha^{-1}) in spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the effect of treatments due to application of mycorrhiza and phosphates has significant influence the soil available P content under naturally Cd contaminated soil. It is well proven that AM Mycorrhiza play key role in mobilizing P in the soil. The stages of the crop growth also have significance influence on the soil available P, this may be due to more availability leads to better uptake by the crop. With the advancement of the crop growing period the P availability improved in the soil.

27.1 Effect of phytoremediation on soil available K (kg ha^{-1}) in spinach crop under cadmium spiked soil

Under Cd spiked soil, only the stages of crop growth has significant influence on the soil available K content. The soil available K content decreases with the advancement of the growing period.

Table 27.2 Effect of phytoremediation on soil available K (kg ha^{-1}) in spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the stages of the crop growth has significant influence on soil available K content under the naturally Cd contaminated soil.

28.1 Effect of phytoremediation on soil available S (mg kg^{-1}) in spinach crop under cadmium spiked soil

In the Cd spiked soil, the treatment effect due to application of AM mycorrhiza and phosphates has significant influence on the soil available S content in the soil. The interaction effect due to treatment and stages of the growing period significant influence. The mycorrhiza may have important role in mineralizing S under Cd spiked soil under the spinach crop rhizosphere.

28.2 Effect of phytoremediation on soil available S (mg kg^{-1}) in spinach crop under naturally cadmium contaminated soil

Unlike Cd spiked soil, the soil available S in spinach crop under naturally contaminated soil has influenced only by the crop growing stage. In the naturally Cd contaminated soil the picture of S availability may be different. With the advancement of the growing period of the plants the soil available S was found less as compared to initial crop growth stage.

29.1 Effect of phytoremediation on soil Cd (ppm) in spinach crop under cadmium spiked soil

In the Cd spiked soil, the soil Cd was significantly influenced by the treatment effects due to application of AM mycorrhiza and SSP application. Mycorrhiza and phosphates (SSP) play important role on influencing soil Cd concentration. The stages of crop growth with respect to phytoremediation also have significant influence on the Soil Cd concentration. Hence, with the advancement of the crop growing period the spinach crop has substantially decreased the soil Cd concentration.

29.2 Effect of phytoremediation on soil Cd (ppm) in spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the soil Cd concentration significantly influence by the treatments with the application mycorrhiza. The stages of the crop growth also has a significant role on soil Cd content and with the advancement of the crop growing period the Cd content of the soil decreases.

30.1 Effect of phytoremediation on DHA (µg TPF g soil^{-1}24h^{-1}) in spinach crop under cadmium spiked soil

The dehydrogenase activity significantly influenced the stages of the crop growing period. In a study by Mandal *et al.,* (2014) reported that the successive growing cycle with Chinese brake fern has improved the soil enzyme activity in arsenic contaminated soil.

30.2 Effect of phytoremediation on DHA (µg TPF g soil^{-1} 24h^{-1}) in spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, here also the stage of the crop growth has great influence on the soil dehydrogenase activity under naturally Cd contaminated soil.

31.1 Effect of phytoremediation on fluorescein di-acetate (*µg* fluorescein g^{-1}) in spinach crop under cadmium spiked soil

In Cd spiked soil, the fluorescein di-acetate activity improved significantly due to application of AM mycorrhiza and phosphates (SSP). The AM mycorrhiza in the rhizosphere improves the influence the microbial activity that may improve the fluorescein di-acetate hydrolysis.

31.2 Effect of phytoremediation on fluorescein di-acetate (*µg* fluorescein g^{-1}) in spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the FDA activity was significantly improved due to application of AM mycorrhiza and phosphates (SSP) in the naturally contaminated soil. In this soil the stages of crop growth has great influence in the FDA activity in soil.

32.1 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in spinach crop under cadmium spiked soil

In Cd spiked soil, the soil respiration has improved significantly due to application of AM mycorrhiza and phosphates (SSP). The AM mycorrhiza in the rhizosphere improves the influence the microbial activity that may improve soil respiration.

32.2 Effect of phytoremediation on soil respiration (mg CO_2-C g^{-1} soil d^{-1}) in spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil exactly same observation found that treatments has great influence on the soil respiration that may be due to AM mycorrhiza on soil microbial activity which in turns improves the soil respiration in naturally Cd contaminated soil.

33.1 Effect of phytoremediation on soil microbial biomass carbon (SMBC) in spinach crop under cadmium spiked soil

Under cadmium spiked soil the soil, the Soil microbial biomass carbon (SMBC) significantly improved by the stages of crop production during the process of phytoremediation. As the Cd accumulation in the plant increase the microbial biomasss in the soil increase due to less toxic effect of Cd for microbial proliferation. Mandal *et al.,* (2014) reported that repeated phytoextraction improves the microbial biomass carbon in the soil.

33.2 Effect of phytoremediation on soil microbial biomass carbon (SMBC) in spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the SMBC was improved due to stages of the crop growth. This observation supported by the study of Mandal *et al.,* 2014.

34.1 Effect of phytoremediation on glomalin (mg kg^{-1}) content in soil during spinach crop under cadmium spiked soil

In the Cd spiked soil, the glomalin content significantly improved due to stage of the crop growth and treatment due to application of AM (mycorrhiza) and SSP.

34.2 Effect of phytoremediation on glomalin (mg kg^{-1}) content in soil during spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the glomalin content in the soil improved due to growing stage of the crop and treatment due to application of AM mycorrhiza and SSP.

35.1 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during spinach crop under cadmium spiked soil

In Cd spiked soil, the population of fungi significantly improved due to application of AM mycorrhiza and phosphates. The effect of treatments and the stages of the crop growth has great influence influencing the microbial growth and population that improves the fungi population in the soil.

35.2 Effect of phytoremediation on fungi (10^{-3} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the fungi population is following same trend with respect to its treatment and stages of the crop growth.

36.1 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during spinach crop under cadmium spiked soil

In Cd spiked soil, the population of bacteria significantly improved due to application of AM mycorrhiza and phosphates. The effect of treatments and the stages of the crop growth has great influence influencing the microbial growth and population that improves the bacterial population in the soil.

36.2 Effect of phytoremediation on bacteria (10^{-5} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the bacterial population is following same trend with respect to its treatment and stages of the crop growth.

37.1 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during spinach crop under cadmium spiked soil

In Cd spiked soil, the population of actinomycetes significantly improved due to application of AM mycorrhiza and phosphates. The effect of treatments has great influence influencing the microbial growth and population that improves the actinomycetes population in the soil.

37.2 Effect of phytoremediation on actinomycetes (10^{-4} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

In the naturally contaminated soil, the actinomycetes population was greatly influenced by the treatment and stages of the crop growth.

38.1 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during spinach crop under cadmium spiked soil

In Cd spiked soil, the population of N fixers significantly improved due to application of AM mycorrhiza and phosphates as well due to crop growing stage. The effect of treatments has great influence influencing the microbial growth and population that improves the N fixers population in the soil.

38.2 Effect of phytoremediation on N-fixer (10^{-3} X cfu) population in soil during spinach crop under naturally cadmium contaminated soil

Similar to Cd spiked soil, the N-fixers population following same trend that may be due to enhanced microbial activity and their population could influence more number of N-fixers in the soil.

39.1 Effect of phytoremediation by mustard on cadmium removal (%) in cadmium spiked soil

In the Cd spiked soil, the effect of treatments and stages of the crop growth significantly improved the Cd removal from the soil. The application of AM mycorrhiza and SSP may solubilise more plant available form of Cd in the soil. With the advancement of the crop growing period the % Cd removal increased that may be due to more Cd accumulation and uptake by the mustard crop.

39.2 Effect of phytoremediation by mustard on cadmium removal (%) in naturally cadmium contaminated soil

In the naturally Cd contaminated s soil, the effect of stages of the crop growth significantly improved the Cd removal from the soil. With the advancement of the crop growing period the % Cd removal increased that may be due to more Cd accumulation and uptake by the crop.

40.1 Effect of phytoremediation by spinach on cadmium removal (%) in cadmium spiked soil

In the Cd spiked soil, the effect of treatments and stages of the crop growth significantly improved the Cd removal from the soil. The application of AM mycorrhiza and SSP may solubilise more plant available form of Cd in the soil. With the advancement of the crop growing period the % Cd removal increased that may be due to more Cd accumulation and uptake by the spinach crop.

40.2 Effect of phytoremediation by spinach on cadmium removal (%) in naturally cadmium contaminated soil

In the naturally Cd contaminated s soil, due to treatments and stages of the crop growth significantly improved the Cd removal from the soil. With the advancement of the crop growing period the % Cd removal increased that may be due to more Cd accumulation and uptake by the crop.

CHAPTER VI

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

A green house study entitled "**Phytoremediation of cadmium contaminated soil by Indian mustard and spinach as influenced by phosphate fertilizer and mycorrhizae**" was conducted during 2014-15 at Indian Institute of Soil Science, Bhopal. In this pot culture experiment two different concentration of Cd was used i.e. one is cadmium spiked soil and another naturally cadmium contaminated soil. The four different treatments were used for this study as follows T1: RDF (Recommended doses of fertilizer); T2: RDF+SSP, T3: RDF+SSP+AM; T4: RDF+SSP+AM with three replications. For this phytoremediation experiment two types of plant were used such as Indian mustard (Pusa Bold) and spinach (All green).

The experimental soil was medium black Vertisol having clay loam texture, pH 7.78, medium in available nitrogen (83.6 kg N/ha), medium in available phosphorus (53.8 kg P/ha), high in available potassium (317.7 kg K/ha), with available Zn (2.25 ppm), Fe (1.475 ppm), Mn (0.684 ppm), Cu (1.818 ppm), content under the soil used for spiking and soil was medium black Vertisol having clay loam texture, pH 6.68, low in available nitrogen (62.7 kgN/ha), medium in available phosphorus (79.8 kg P/ha), high in available potassium (453 kg K/ha), with available Zn (0.823 ppm), Fe (1.845 ppm), Mn (1.457 ppm), Cu (2.40 ppm), content under the naturally cd contaminated soil mg/kg respectively. Variety was sown for Indian mustard and spinach respectively.

Various chemical parameters were studied during this study such as plant N, P, K, S and heavy metal Cd content in various parts of plant such as shoot, root and grain for mustard and shoots and roots of spinach. The soil biological parameters such as soil dehydrogenase activity, Fluorescein Di-acetate hydrolysis, soil respiration, microbial biomass carbon, glomalin content, microbial counts (Fungi, Bacteria, actinomycetes), N-fixers etc. The effect of phytoremediation by the mustard and spinach on total Cadmium removal (%) also analysed in this study.

Summary

1. The application of recommended doses of fertilizer (RDF)+ single super phosphate (SSP) + AM (arbuscular mycorrhiza) is found best treatment for improving the plant nutrition status (N,P,K, S) and improving soil biological properties such as dehydrogenase, fluorescin di-acetate. Among the treatment, T4 was found superior than the rest of the treatment.

2. Mycorrhizal inoculation along with phosphate application (SSP) improved the soil available nutrient content and Cd bioavailability to the plants.

3. The effect of phytoremediation with respect to the stage of the growth period has great influence on the nutrient content, soil available nutrient content (N, P, K & S) and soil biological properties (dehydrogenase, fluorescin di-acetate, soil respiration) and glomalin content and population of soil bacteria, fungi and actinomycetes, N-fixers in the soil.

4. The Cd removal % increasing due to its growing period, the degree of Cd removal was found higher under the Cd spiked soil as compared to naturally contaminated soil. The treatment T4 was found most efficient to remove Cd from the contaminated soil.

5. Among the treatments, T1 has very little significance on nutrient content followed by T2<T3<T4.

6. With the advancement of the growing period there is a greater removal of Cd both from the naturally Cd contaminated soil and Cd spiked soil.

Conclusion

(1) On the basis of present investigation, it may be concluded that the application of RDF+ AM + SSP (T_4) proved beneficial for better nutrient acquisition by the crop both in mustard and spinach. There was a improvement in soil biological properties, microbial population and cadmium removal by the crop.

(2) In case of growth stages of mustard and spinach, the latter stage of growing indicates better soil microbiological performance and improvement of microbial population.

(3) In the cadmium spiked soil, the mustard performed better the cadmium removal by the crop as compared to spinach whereas in naturally Cd contaminated soil spinach crop was found better than the mustard crop.

Suggestions for further work

The edible crops like mustard and spinach used for phytoremediation may be dangerous for its consumption point of view. The potential for removal of Cd by these mustard and spinach in a single growing season is not optimum, hence repeated cycles of crop growth with different season may revealed better picture from the Cd contaminated soil. There may be scope for indentifying plants growing luxuriantly in the various metals contaminated site and also test for its maximum potential to decontaminate the contaminated soil. By performing the pot culture study and the research study should be elaborated at the field site remediation. Further, in a mixed contaminated soil with organic and inorganic pollutants, the remediation through roots-microbes interaction also demands attention.

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APPENDICES

Appendix 1.1 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in mustard shoot under cadmium spiked soil

	DF	N Content	P Content	K Content	S Content
Fertilizers	3	0.204516	0.024893	0.182455	0.12045
Stage (S)	1	0.415502	0.053098	3.659766	0.282477
F x S	3	0.013082	0.005104	0.001117	0.024448
Error	16	0.028422	0.004276	0.228945	0.022239

Appendix 1.2 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in mustard shoot under naturally cadmium contaminated soil

	DF	N Content	P content	K Content	S Content
Fertilizers	3	0.289085	0.019923	0.514811	0.120516
Stage (S)	1	2.118852	0.325361	4.910531	0.497327
F x S	3	0.000946	0.001183	0.025402	0.028538
Error	16	0.132606	0.326545	0.311671	0.036434

Appendix 1.3 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in mustard root under cadmium spiked soil

	DF	N Content	P Content	K Content	S Content
Fertilizers	3	0.520588	0.005215	0.124299	0.009724
Stage (S)	1	0.059714	0.19585	4.757942	0.259952
F x S	3	0.047483	0.002731	0.007333	0.00383
Error	16	0.107198	0.012753	0.298746	0.016965

Appendix 1.4 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in mustard root under naturally cadmium contaminated soil

	DF	N Content	P Content	K Content	S Content
Fertilizers	3	0.431511	0.001554	0.158799	0.06993
Stage (S)	1	0.010599	0.268152	5.672593	0.024943
F x S	3	0.1712	0.000581	0.03438	0.028661
Error	16	0.032762	0.016868	0.360983	0.006933

Appendix 1.5 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in mustard grain under cadmium spiked soil

	DF	N Content	P Content	K Content	S Content
Fertilizers	3	1.94591	0.588953	0.160396	0.140107
Stage (S)	1	21.09386	4.54052	7.987988	0.074316
F x S	3	0.356914	0.044679	0.021222	0.023862
Error	16	1.385288	0.29216	0.503228	0.009119

Appendix 1.6 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in mustard grain under naturally cadmium contaminated soil

	DF	N Content	P Content	K Content	S Content
Fertilizers	3	1.026185	0.244225	0.046229	0.406686
Stage (S)	1	21.62251	12.08713	13.66701	0.43094
F x S	3	0.058306	0.014475	0.001869	0.260707
Error	16	1.362339	0.75816	0.854539	0.075816

Appendix 1.7 Mean sum of square of phytoremediation on soil available N, P, K (kg ha^{-1}), S (mg kg^{-1}), and Cd (ppm) in mustard crop under cadmium spiked soil

	DF	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	S (mg kg^{-1})	Cd (ppm)
Fertilizers	3	1532.673	889.0844	1656.139	5.46	552.7968
Stage (S)	1	16852.98	160.1253	2196.507	1.04	277.236
F x S	3	12.47888	31.28704	127.1648	3.9	7.804615
Error	16	1055.651	15.87415	161.1251	7.38	18.79062

Appendix 1.8 Mean sum of square of phytoremediation on soil available N, P, K (kg ha^{-1}), S (mg kg^{-1}), and Cd (ppm) in mustard crop under naturally cadmium contaminated soil

	DF	N (kg ha^{-1})	P(kg ha^{-1})	K (kg ha^{-1})	S(mg kg^{-1})	Cd (ppm)
Fertilizers	3	2718.583	837.6598	1331.528	0.00015	0.019082
Stage (S)	1	28993.73	1064.023	1564.289	2.3	0.095004
F x S	3	653.9503	199.6888	150.9984	6.18	0.002182
Error	16	1934.724	103.9431	126.0803	2.6	0.006347

Appendix 1.9 Mean sum of square of phytoremediation on dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$), Fluorescein Di-acetate (*µg* Fluorescin g^{-1}), Soil respiration (mg CO_2-C g $soil^{-1}$ d^{-1}), Soil microbial biomass carbon (SMBC) and glomalin (mg kg^{-1}) in mustard crop under cadmium spiked soil

	DF	D.H.A. (µg TPF g^{-1} soil $24h^{-1}$)	F.D.A. (*µg* Fluorescin g^{-1})	S.R. (mg Co_2-C g $soil^{-1}$ d^{-1})	S.M.B.C.	Glomalin (mg kg^{-1})
Fertilizers	3	841.4356	42.02651	0.001286	0.127568	90.12452
Stage (S)	1	6942.938	475.2485	1.5E-06	0.6534	418.4552
F x S	3	37.75361	17.30361	3.57E-05	0.064648	4.655538
Error	16	441.0124	32.94746	6.79E-06	0.052959	27.02637

Appendix 1.10 Mean sum of square of phytoremediation on dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$), Fluorescein Di-acetate (*µg* Fluorescin g^{-1}), Soil respiration (mg Co_2-C g $soil^{-1}$ d^{-1}) and Soil microbial biomass carbon (SMBC) in mustard crop under naturally cadmium contaminated soil

	DF	D.H.A. (µg TPF g^{-1} soil $24h^{-1}$)	F.D.A. (*µg* Fluorescin g^{-1})	S.R. (mg Co_2-C g $soil^{-1}$ d^{-1})	S.M.B.C.	Glomalin (mg kg^{-1})
Fertilizers	3	2058.895	34.03478	0.000936	0.011494	170.5333
Stage (S)	1	59112.11	78.85764	0.002243	1.03335	713.2786
F x S	3	97.2196	1.641763	0.000103	0.004294	7.277347
Error	16	3712.736	5.236433	0.00016	0.06539	45.94442

Appendix 1.11 Mean sum of square of phytoremediation on fungi (10^{-3} X cfu), bacteria (10^{-5} X cfu), actinomycetes **(10^{-4} X cfu) and N-fixer (10^{-3} X cfu) in mustard under cadmium spiked soil**

	DF	Fungi (10^{-3} X cfu)	Bacteria (10^{-5} X cfu)	Actinomycetes (10^{-4} X cfu)	N-fixer (10^{-3} X cfu)
Fertilizers	3	3.967593	22.03704	22.70833	80.55556
Stage (S)	1	0.115741	15.57407	0.560185	244.9074
F x S	3	0.276235	2.425926	2.436728	0.549383
Error	16	0.059028	1.428241	0.491898	15.40972

Appendix 1.12 Mean sum of square of phytoremediation on fungi (10^{-3} X cfu), bacteria (10^{-5} X cfu), actinomycetes **(10^{-4} X cfu) and N-fixer (10^{-3} X cfu) in mustard crop under naturally cadmium contaminated soil**

	DF	**Fungi (10^{-3} X cfu)**	**Bacteria (10^{-5} X cfu)**	Actinomycetes **(10^{-4} X cfu)**	**N-fixer (10^{-3} X cfu)**
Fertilizers	3	7.263889	22.75772	25.83333	71.64198
Stage (S)	1	2.893519	51.04167	26.74074	153.3519
F x S	3	0.313272	8.486111	1.888889	4.648148
Error	16	0.239583	4.78125	2.025463	10.45602

Appendix 1.13 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in spinach plant crop under cadmium spiked soil

	DF	**N Content**	**P Content**	**K Content**	**S Content**	**Cd Content**
Fertilizers	3	3.47	2.03	167.48	1.75	114296
Stage (S)	2	1.73	0.02	2.06	0.10	1736.03
F x S	6	1.78	1.03	84.09	0.90	57280.6
Error	24	0.00	0.00	0.01	0.00	200.89

Appendix 1.14 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) content (%) in spinach plant crop under naturally contaminated soil

	DF	**N Content**	**P Content**	**K Content**	**S Content**	**Cd Content**
Fertilizers	3	5.77	3.64	185.38	3.73	52.99
Stage (S)	2	3.10	0.05	5.60	1.26	6.73
F x S	6	2.96	1.86	93.03	2.55	29.13
Error	24	0.00	0.00	0.00	0.45	0.80

Appendix 1.15 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) in spinach root under cadmium spiked soil

	DF	**N Content**	**P Content**	**K Content**	**S Content**	**Cd Content**
Fertilizers	3	0.007551	0.014043	0.093847	0.008943	20.06444
Stage (S)	1	0.007859	0.85992	12.97716	0.190129	3901.5
F x S	3	0.000519	0.001882	0.003105	0.001835	146.5978
Error	16	0.000589	0.054098	0.811655	0.012227	271.3308

Appendix 1.16 Mean sum of square of phytoremediation on N, P, K, S, and Cd content (%) content (%) in spinach root under naturally cadmium contaminated soil

	DF	**N Content**	**P Content**	**K Content**	**S Content**	**Cd Content**
Fertilizers	3	0.005783	0.030411	0.15166	0.00921	0.712778
Stage (S)	1	0.002739	0.994024	19.96915	0.13245	19.08167
F x S	3	0.000586	0.005281	0.033214	0.002405	0.152778
Error	16	0.000281	0.063117	1.2543	0.008729	1.22125

Appendix 1.17 Mean sum of square of phytoremediation on soil available N, P, K (kg ha^{-1}), S (mg kg^{-1}), and Cd (ppm) in spinach crop under cadmium spiked soil

	DF	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	S (mg kg^{-1})	Cd (ppm)
Fertilizers	3	2561.165	1333.587	1710.078	0.000726	1367.45
Stage (S)	1	1397.547	1119.77	9529.729	1.75E-05	47.54535
F x S	3	34.35517	8.302795	65.42044	0.000478	2.204639
Error	16	93.78831	71.54242	607.8744	9.08E-05	3.384954

Appendix 1.18 Mean sum of square of phytoremediation on soil available N, P, K (kg ha^{-1}), S (mg kg^{-1}), and Cd (ppm) in spinach crop under naturally cadmium contaminated soil

	DF	N (kg ha^{-1})	P(kg ha^{-1})	K (kg ha^{-1})	S(mg kg^{-1})	Cd (ppm)
Fertilizers	3	2029.927	1948.679	1749.888	0.002428	0.008137
Stage (S)	1	736.6693	1802.972	8786.027	0.0138	0.027337
F x S	3	310.2456	413.1629	235.6181	0.000492	0.001049
Error	16	104.2129	190.1538	593.3051	0.000955	0.001905

Appendix 1.19 Mean sum of square of phytoremediation on dehydrogenase activity (µg TPF g^{-1} soil 24h^{-1}), Fluorescein Di-acetate (*µg* Fluorescin g^{-1}), Soil respiration (mg Co_2-C g soil^{-1} d^{-1}), Soil microbial biomass carbon (SMBC) and glomalin (mg kg^{-1}) in spinach crop under cadmium spiked soil

	DF	D.H.A. (µg TPF g^{-1} soil 24h^{-1})	F.D.A. (*µg* Fluorescin g^{-1})	S.R. (mg Co_2-C g soil^{-1} d^{-1})	S.M.B.C.	Glomalin (mg kg^{-1})
Fertilizers	3	1031.865	62.84829	0.00177	0.017206	86.52882
Stage (S)	1	17062.08	0.216614	0.000113	0.396294	304.0981
F x S	3	39.03637	1.553038	0.000149	0.000598	35.15946
Error	16	1073.699	0.304733	3.49E-05	0.024881	25.59853

Appendix 1.20 Mean sum of square of phytoremediation on dehydrogenase activity (µg TPF g^{-1} soil 24h^{-1}), Fluorescein Di-acetate (*µg* Fluorescin g^{-1}), Soil respiration (mg Co_2-C g soil^{-1} d^{-1}), Soil microbial biomass carbon (SMBC) and glomalin (mg kg^{-1}) in spinach crop under naturally cadmium contaminated soil

	DF	D.H.A. (µg TPF g^{-1} soil 24h^{-1})	F.D.A. (*µg* Fluorescin g^{-1})	S.R. (mg Co_2-C g soil^{-1} d^{-1})	S.M.B.C.	Glomalin (mg kg^{-1})
Fertilizers	3	1248.408	87.93626	0.001665	0.053128	254.4703
Stage (S)	1	53572.05	126.858	2.02E-05	1.723776	1067.862
F x S	3	202.4868	32.62627	5.22E-05	0.011152	38.57999
Error	16	3386.219	14.04605	1.1E-05	0.109827	73.9751

Appendix 1.21 Mean sum of square of phytoremediation on fungi (10^{-3} X cfu), bacteria (10^{-5} X cfu), actinomycetes **(10^{-4} X cfu) and N-fixer (10^{-3} X cfu) in spinach crop under cadmium spiked soil**

	DF	Fungi (10^{-3} X cfu)	Bacteria (10^{-5} X cfu)	Actinomycetes (10^{-4} X cfu)	N-fixer (10^{-3} X cfu)
Fertilizers	3	3.462963	14.52315	35.12963	114.4012
Stage (S)	1	6	2.449074	2.240741	196.463
F x S	3	0.432099	0.523148	10.73457	17.3642
Error	16	0.456019	0.251157	2.152778	15.53472

Appendix 1.22 Mean sum of square of phytoremediation on fungi (10^{-3} X cfu), bacteria (10^{-5} X cfu), actinomycetes **(10^{-4} X cfu) and N-fixer (10^{-3} X cfu) in spinach crop under naturally cadmium contaminated soil**

	DF	**Fungi 10^{-3} X cfu)**	**Bacteria (10^{-5} X cfu)**	Actinomycetes **(10^{-4} X cfu)**	**N-fixer (10^{-3} X cfu)**
Fertilizers	3	7.979938	24.89506	32.9429	64.2284
Stage (S)	1	15.04167	266.6667	11.11574	236.463
F x S	3	0.708333	8.82716	3.695988	1.364198
Error	16	1.072917	18.32176	1.387731	15.03472

Appendix 1.23 Mean sum of square of phytoremediation by mustard and spinach on cadmium removal (%) in Cd spiked and Cd contaminated soil

		Cadmium spiked soil		**Cadmium contaminated soil**	
	DF	**Mustard**	**Spinach**	**Mustard**	**Spinach**
Fertilizers	3	547.7133	1354.875	47.16095	20.1118
Stage (S)	1	274.6866	47.10812	234.8024	67.56452
F x S	3	7.732843	2.184365	5.392667	2.591638
Error	16	18.61782	3.353826	15.68628	4.708715

VITA

The author of this thesis Mr. Sandeep Karode s/o Shri Ganesh Karode and Smt. Dhanu bai was born on 21th November 1988 at Maheshwar, Teh- Maheshwar, Dist- Khargone (MP). He passed his higher secondary school certificate examination from Govt. High Secondary School, Maheshwar with 69.66%. He joined the College of Agriculture, in university of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (MP) in the year 2009-10 and successfully completed the degree of B.Sc.(Ag) during the year 2012-13 with 7.02 OGPA at 10 point scale.

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