

Qana Anajaa sara [va ki vaW tqa paak t%aNevalBarl Qatu] dyaNa pr jaD,
gaat sal kRa nayaDagayad gaDnakaBa ka pBaBa

**Effect of Root-knot Nematode, *Meloidogyne graminicola* on
the Nutrients and Heavy Metal Uptake and Growth of Rice,
*Oryza sativa***

M. Venkatesan



**DIVISION OF NEMATODOLOGY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI – 110012**

2014

**Effect of Root-Knot Nematode, *Meloidogyne graminicola* on
the Nutrients and Heavy Metal Uptake and Growth of Rice,
*Oryza sativa***

A Thesis

by

M. Venkatesan

**Submitted to the Faculty of Post-Graduate School,
Indian Agricultural Research Institute, New Delhi,**

in partial fulfilment of the requirements

for the award of the degree of

DOCTOR OF PHILOSOPHY

In

Nematology

2014

Approved by:

Chairperson: Dr. H. S. Gaur

Members: Dr. Anil Sirohi

Dr. Pankaj

Dr. Pratibha Sharma

Dr. Archana Sachdev

**INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-110 012**

Dr. H. S. Gaur
Former Dean & Jt. Director (Edu)

Presently: Vice Chancellor, SVPUA&T, Meerut, U.P.

CERTIFICATE

This is to certify that the thesis entitled, “**Effect of Root-Knot Nematode, *Meloidogyne graminicola* on the Nutrients and Heavy Metal Uptake and Growth of Rice, *Oryza sativa*”** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of **Doctor of Philosophy in Nematology**, embodies the results of *bonafide* research work carried out by **Mr. M. Venkatesan** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

I further certify that any help received during the course of investigation as well as source of information have been duly acknowledged.

Date: 15 February, 2014

(H. S. Gaur)

Place: New Delhi

Chairman, Advisory Committee

DEDICATED TO MY FAMILY

ACKNOWLEDGEMENT

I am obliged to express my deep sense of gratitude and indebtedness to Dr H. S. Gaur, former Dean and Joint Director (Education), IARI, New Delhi, presently, Vice Chancellor, SVPUA&T, Meerut, and Chairman of my Advisory Committee for his meticulous guidance, sustained encouragement, undivided dedication, constructive criticism and imparting his enormous knowledge to build my strengths not only during the course of these investigations but also in the preparation of the manuscript and research papers. Despite his multidimensional responsibilities, he most affectionately extended his kind help, cooperation and encouragement. It was a great opportunity and a special privilege for me to be his student.

I wish to express my gratitude to the members of my Advisory committee, Drs. Anil Sirohi and Pankaj, both Principal Scientists, Division of Nematology and Pratibha Sharma Professor, Division of Plant Pathology; Archana Sachdev, Principal Scientist, Division of Bio-chemistry and S.P. Datta, Senior Scientist, Division of Soil Science and Agricultural Chemistry, IARI, New Delhi for their valuable guidance, perpetual support, ever-willing assistance and guidance given to me throughout this endeavour.

It is my privilege to acknowledge my indebtedness to Dr. (Mrs.) Uma Rao, Head and Dr. (Mrs.) Sudershan Ganguly, Professor, and Dr. A. K. Ganguly, Former Head, and Dr. D. Prasad, Former Professor, Division of Nematology, IARI, New Delhi in my Ph.D. studentship at IARI, New Delhi for providing facilities and for their constant support and constructive suggestions.

I cannot forget to express my obligation and appreciation for all esteemed scientists and Technical, administrative and supporting staff of the Division of Nematology, for their help and encouragement throughout my study at IARI. I especially extend my regards to Drs. Anju Kamra, Sharad Mohan, Gautam Chawla and H. K. Sharma for their encouragement, help and valuable suggestions during the course of investigation. I also express my sincere thanks to the Heads of the Divisions

of Plant Pathology, Bio-chemistry and Soil Science and Agricultural chemistry, IARI, for providing necessary facilities throughout my studies.

I express my deep sense of gratitude to my parents and sisters and my friends, Aarthi and Surya shree for their continued love and support throughout my life.

It is a matter of privilege for me to express my whole hearted thanks to my beloved friends Mardu durai, Ganesa moorthi, Sella perumal and Chandramani whose moral support, advice and assistance helped me in all walks of my life.

I extend my thanks to all the staff of PG School, IARI for their guidance and help in completing all the formalities for my study.

I am grateful to the Director, IARI, New Delhi, for providing me the necessary facilities to carry out my research work at this Institute.

Last but not the least I gratefully acknowledge the financial assistance as Senior Fellowship received from the Indian Council of Agricultural Research, New Delhi and IARI, New Delhi.

Place: IARI, New Delhi

M.Venkatesan

Date: 15 Feb., 2014

CONTENTS

CHAPTER	TITLE	PAGE No
1	INTRODUCTION	1
2	BACKGROUND	5
3	MATERIALS & METHODS	20
4	RESULTS	23
4.1	RESEARCH PAPER I	23
4.2	RESEARCH PAPER II	33
4.3	RESEARCH PAPER III	41
4.4	RESEARCH PAPER IV	48
5	GENERAL DISCUSSION	57
6	SUMMARY & CONCLUSION	60
	ABSTRACT (ENGLISH & HINDI)	I-III
	BIBLIOGRAPHY	IV-XVII

LIST OF TABLES

TABLE No	TITLE	PAGE AFTER
4.1.1	Effect of Arsenic and root-knot nematode, <i>Meloidogyne graminicola</i> on the macro nutrients, NPK in shoot of rice cv.Pusa 1121	27
4.1.2	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Arsenic uptake by rice cv. Pusa 1121	27
4.1.3	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Arsenic uptake by rice cv. Pusa 1121	28
4.1.4	Effect of Arsenic and root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 1121	29
4.2.1	Effect of Lead and root-knot nematode, <i>Meloidogyne graminicola</i> on the macronutrients, NPK in shoot of rice cv. Pusa 1121	36
4.2.2	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Lead uptake by rice cv. Pusa 1121	37
4.2.3	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Lead uptake by rice cv. Pusa 1121	38
4.2.4	Effect of Lead and root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 1121	38
4.3.1	Effect of Cadmium and root-knot nematode, <i>Meloidogyne graminicola</i> on the macro nutrients, NPK in shoot of rice cv.Pusa 1121.	44
4.3.2	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Cadmium uptake by rice cv. Pusa 1121	45
4.3.3	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Cadmium uptake by rice cv. Pusa 1121	45
4.3.4	Effect of Cadmium and root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 1121	45
4.4.1	Effect of varying population densities of root-knot nematode, <i>Meloidogyne graminicola</i> on macro-nutrients uptake of rice cv. Pusa 1121 (shoot) in field	51
4.4.2	Effect of varying population densities of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metals uptake of rice cv. Pusa 1121 in field	52
4.4.3	Effect of varying population densities of root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 1121 in field	53

LIST OF FIGURES

FIGURE No.	TITLE	AFTER PAGE
4.1.1	Effect of <i>Meloidogyne graminicola</i> @ 2 J ₂ cm ⁻² on Arsenic content in root and shoot of 20 day rice plants grown in soil from West Bengal artificially contaminated Arsenic	27
4.1.2	Effect of Arsenic and root-knot nematode, <i>Meloidogyne graminicola</i> on the macro nutrients, NPK in shoot of rice cv. Pusa 1121	27
4.1.3	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Arsenic uptake by rice cv. Pusa 1121, 45 days after transplanting	28
4.1.4	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Arsenic uptake by rice cv. Pusa 1121, 110 days after transplanting	28
4.1.5	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 1121	29
4.2.1	Effect of Lead and root-knot nematode, <i>Meloidogyne graminicola</i> on the macronutrients, NPK in shoot of rice cv. Pusa 1121	36
4.2.2	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Lead uptake by rice cv. Pusa 1121, 45 days after transplanting	37
4.2.3	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Lead uptake by rice cv. Pusa 1121, 110 days after transplanting	38
4.2.4	Effect of Lead and root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 112	38
4.3.1	Effect of Cadmium and root-knot nematode, <i>Meloidogyne graminicola</i> on the macronutrients, NPK in shoot of rice cv. Pusa 1121	44
4.3.2	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Cadmium uptake by rice cv. Pusa 1121, 45 days after transplanting	45
4.3.3	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metal, Cadmium uptake by rice cv. Pusa 1121, 110 days after transplanting	45
4.3.4	Effect of root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 1121	45
4.4.1	Effect of varying population densities of root-knot nematode, <i>Meloidogyne graminicola</i> on macro-nutrients uptake of rice cv. Pusa 1121 (shoot) in field	51
4.4.2	Effect of varying population densities of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metals uptake of rice cv. Pusa 1121 in field	52
4.4.3	Effect of varying population densities of root-knot nematode, <i>Meloidogyne graminicola</i> on heavy metals	52

	uptake of rice cv. Pusa 1121 in field	
4.4.4	Effect of varying population densities of root-knot nematode, <i>Meloidogyne graminicola</i> on plant growth of rice cv. Pusa 1121 in field	53

ABBREVIATIONS

Ppm	Parts per million
H	Hours
Cm	Centimetre
G	Gram
°C	Degree Celsius
%	Per cent
Fig	Figure
J2	Second stage juvenile
Conc.	Concentration
AAS	Atomic Absorption Spectrometry
Mg	Microgram
Dm	Decimetre
UV	Ultra Violet
v/v	Volume/Volume
DAT	Days After Transplanting
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
Na ₂ HAsO ₄ . 7 H ₂ O	Sodium arsenate dibasic heptahydrate
Pb (NO ₃) ₂	Lead nitrate
Cd (NO ₃) ₂ .4 H ₂ O	Cadmium nitrate

Rice (*Oryza sativa* L.), is a staple food in 17 countries in Asia and the Pacific, nine countries in North and South America and eight countries in Africa thus forming the staple diet of over 2.7 billion people. It is grown over about 150 million ha, producing 573 million tonnes paddy with an average productivity of 3.83 t/ha (Pokharel *et al.*, 2007). Rice provides 20 per cent of the world's dietary energy supply. Its cultivation is of immense importance in Asia, where more than 90% of global rice is produced and consumed. India is the largest rice growing country, while China is the largest producer of rice which highlights the lower productivity level in India.

Rice is grown in four major types of ecosystems: irrigated (72%), rainfed low land (19%), upland (5%), and deepwater/tidal wetland (4%), (Anonymous, 1989). Rice proteins are of very high quality as compared to other food crops. It has a relatively favourable amino acid composition with a high proportion of lysine and a high protein digestibility. Brown rice not only has higher protein content, but also a higher proportion of lysine than milled rice.

Rice is mostly considered a starchy staple food, which provides a large portion (sometimes up to 90% in Asia) of dietary energy. Brown rice contains about 75-85 percent carbohydrates, and milled rice even around 90 percent. Rice varieties differ in the proportion of the two starchy fractions: amylose consists of linear linked glucose molecules and amylopectine is composed of glucose molecules with branched links. The starch of so called waxy rice varieties consists of amylopectine only. These varieties absorb less water upon cooking and have a sticky texture. On the other hand, rice varieties with amylose content of more than 25% absorb more water and have a fluffy texture after cooking.

The root-knot nematode, *Meloidogyne graminicola* (Golden and Birschfield, 1965), is a pest of international importance to rice crop around the world. It is known to infect and cause serious damage to cereals, especially rice, in many countries causing estimated yield losses of between 20% and 80% (Arayarungsarit, 1987; Netscher and Erlan, 1993; Soriano Reversat, 2003; Padgham *et al.*, 2004 and Pokharel *et al.*, 2007). Gaur *et al.*, (1993) reported the occurrence of two species

M.graminicola and *M.triticoryzae* in rice-wheat cropping systems in north India where much of Indian basmati is cultivated. In India the nematodes are known to cause damage especially in rice nurseries and upland rice crops in the eastern and southern coastal region but are now prevalent in most rice growing regions of the country. The wheat crop served as a green between the two rice crops thus increasing the infestation levels rapidly.

M. graminicola causes economic losses in rice that vary depending on the agro ecosystem in which the rice crop is grown. Losses of 20% have been documented for rice cultivars IR 29 and IR 74 under intermittent flooding (Plowright, and Bridge, 1990), 30% in semi deep water rice (Prasad *et al.*, 1990), and of 70% under saturated conditions when the nematode infects young susceptible seedlings at transplanting (Prot *et al.*, 1995). Jairajpuri and Baqri, 1991 reported grain yield losses from 16 to 32%. The nematode has adapted to its environment in that its egg masses can remain viable in waterlogged soil for 14 months or more (Anonymous, 1989). Once the soil dries, the second stage juveniles become active and enter the roots of the succeeding susceptible crops. High population density of *M. graminicola* second stage juveniles can cause seedling mortality, reduction in plant height, tiller number and grain yield loss up to 98% (Plowright and Bridge, 1990; Prasad *et al.*, 1990). The root-knot nematodes infect the growing regions of the root inducing hypertrophy and hyperplasia of the vascular and cortical cells as a result of a biochemical interaction between nematode saliva and tryptophan, the presence indole acetic acid, Schoeneweiss, 1978. The morphological and chemical changes in root system may hamper the uptake of macro- and micro-nutrients and water from soil. This may results in deficiencies of the vital ingredients in the shoot reducing growth and yield.

Soil management practices can impact the physiological susceptibility of the crop plants to nematode pests by either affecting the resistance of individual plant to attack or by altering plant acceptability to pests. Some studies have also documented how the shift from organic soil management to chemical fertilizers has increased the potential of certain pests and diseases to cause economic losses. Although researches on this area have been done for many years, most of the activities mainly focused on impacts of nitrogen on major pests, such as rice blast, stem borers and BPH. As rice production system is changing due to the development of new technology and yield is getting higher, the interactions between nutrition management and nematodes are becoming more complicated and important. The grain nutrient content of rice

depends on a chain of events such as translocation of nutrients from root to shoot, physiological parameters like photosynthesis, plant growth and water use efficiency and transpiration and respiration etc., sufficient uptake, translocation and utilization of nutrients are required for production of good quality seed. Plant parasitic nematodes are root-feeding pests that greatly reduce the development of plant roots. Damaged root systems are less efficient at water and nutrient uptake. Consequently, plant that suffers nematode damage may require more water and fertilizer to maintain a desired appearance of growth and yield.

Crop losses are classified as qualitative or quantitative. In crops meant for direct consumption the quantitative losses are usually evaluated and rarely quality traits are also considered. These criteria are inadequate for an assessment of the impact of nematodes in seed production programmes and in varietal evaluation trials. It is easy to determine the extent of loss in seed grain production, but little is known of the losses due to detrimental effects of plant parasitic nematodes on the quality, viability and vigour of the seed. Recent studies have shown significant reduction in vigour of rice seed grown on *M. graminicola* infested soil (Patil, 2013).

Nematodes not only affect the quantity of seed produced but may also influence the nutritional parameters, quality traits and performance of the crop raised using seed produced in nematode infested field compared to that in an uninfested field. An assessment of these effects is essential for evaluating the impact of plant parasitic nematodes on seed industry.

With increasing soil pollution with industrial effluents and other sources, the heavy metal accumulation and uptake by crops are likely to be increased. The concentration of heavy metals such as arsenic, lead, cadmium etc. in the soil has been reported to be high in certain parts of the country. These heavy metals may be taken up by the root in plants to edible parts including seeds. This is potentially harmful to human and animal health. Sufficient information is not available on how nematodes infecting the roots affect uptake and retention in the plant roots and translocation in the plant. Therefore, the present investigations were proposed to compare effect of the different population densities of *M. graminicola* on nutrient uptake, heavy metal uptake and growth of rice plant. A variety of rice cv. Pusa 1121 was taken for the purpose with the following objectives:

1. To study the effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of macro-nutrients, NPK and plant growth of rice.
2. To study the effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of heavy metals, Arsenic, Cadmium and Lead and growth of rice.
3. To find out whether nematode infection can influence the health hazard due to heavy metal uptake by rice cultivated in naturally heavy metal rich or polluted soils.

Nematodes are well known to cause reduction in the yields of various crops. Crop losses are classified as qualitative or quantitative. In crops meant for direct consumption the quantitative losses are usually evaluated and rarely quality traits are also considered. These criteria are inadequate for an assessment of the impact of nematodes in seed production programmes and in varietal evaluation trials. It is easy to determine the extent of loss in seed grain production, but little is known of the losses due to detrimental effects of plant parasitic nematodes on the quality, viability and vigour of the seed. Nematodes not only affect the quantity of seed produced but may also influence the nutritional parameters, quality traits and performance of the crop raised using seed produced in nematode infested field compared to that in an uninfested field. An assessment of these effects is essential for evaluating the impact of plant parasitic nematodes on seed industry. With increasing soil pollution with industrial effluents and other sources, the heavy metal accumulation and uptake by crops are likely to be increased. The concentration of heavy metals such as Arsenic, Lead, Cadmium and Mercury etc. in the soil has been reported to be high in certain plant parts including seeds. These heavy metals may be taken up by the root in plants to edible parts including seeds. This is potentially harmful to human and animal health. Most of the published reports focused mainly on the uptake of nutrients in the rice plants under the influence of root-knot nematodes and uptake of heavy metals in rice plant irrigated with heavy metals contaminated water and soil. There is a serious gap in our knowledge of any direct relation between heavy metal uptake by rice and root-knot nematodes. Hence these investigations were carried out to determine the concentration of heavy metals taken up by rice crop in the presence of varying population densities of root-knot nematodes in natural condition and artificially contaminated soil.

2.1. Effect of nematodes on nutrient uptake and translocation

When nematodes infect plants, nutrient levels may be decreased selectively increased and/or their distribution between the different plant parts altered which could result in a change in host physiology. An adequate supply and distribution of nutrient elements enable normal plant growth. When plants are infected by

nematodes, such as *Meloidogyne*, their nutrient and physiological status change (Bergeson, 1966).

Studies on the uptake of water as affected by nematodes generally indicate that nematode injury to root system decreases both water and solute absorption and their translocation to the shoots. Different inoculum levels of *Meloidogyne incognita* significantly decreased water absorption capacity of tomatoes compared with that of healthy plants (Alam and Saxena, 1975a). Similar effects were reported for cauliflower and cabbage infected with *Tylenchorhynchus brassicae* (Alam and Saxena, 1975b) and for tomato infected with *M. javanica* (Meon *et al.*, 1978). The influence of nematodes on water uptake and on other physiological processes was shown by Evans *et al.* (1977) who suggested that increased calcium per unit of water transpired in nematode infected plants may be an indicator of direct or indirect nematode damage.

2.1.1 Macronutrient uptake and translocation

Oteifa (1952) reported a decrease in percent root nitrogen, phosphorous and potassium in *Trichodorus christiei* infected lima bean. Oteifa and Elgindi (1962) postulated that the differential uptake of nutrient elements in nematode infected plants could be due to the absorption of some elements by the parasites during their developmental stages within the host. In the *M. javanica* infected tomato plants, phosphorous accumulated in their roots reduced and showed translocation to vegetative organ. While Hunter (1958) found that increased concentration of nitrogen, phosphorus, and potassium in the roots of *M. incognita* infected tomato plants. Using split root technique Bergeson (1966) proved that *M. incognita* infected tomato plants had more nitrogen, phosphorus and potassium in galled tissues than in non-galled tissue.

Trudgill *et al.* (1975) however, found a decrease in percent nitrogen, phosphorous and potassium in potato roots infected with *Globodera rostochiensis*. Bergeson (1966) studied the mobility of several minerals in tomato plant infected with *M. incognita* and concluded that the nematode caused an increase in their concentration in the roots but not in the leaves indicating that transport to the shoot was adversely affected.

There was increase in the concentration of nitrogen, phosphorus and potassium in pepper roots infected with *M. incognita acrita* but not in the shoots

(Maung and Jenkins, 1959; Shafiee and Jenkins, 1963). Dos santos and Ferraz (1981) found that *M. exigua* infested coffee plant absorbed lesser nitrogen and the plant height and root dry weight were decreased with increasing inoculum levels. The root-knot nematode, *M. konaensis* on coffee reduced nitrate and ammonia uptake rates by 63% and 54%, respectively and the migratory nematode, *Pratylenchus coffeae* reduced nitrate uptake rate by 56% and ammonia uptake rate by 24% (Vast *et al.* 1998). Ramakrishnan and Rajendran (1999) found that in papaya roots infected with single and concomitant populations of *M. incognita* and *Rotylenchus reniformis*, there were significant increases in percent nutrient content of nitrogen (12.9) and phosphorous (26.1) in root over the uninoculated control. The concentration of phosphorus and potassium decreased in the coffee roots in 3-month old plants transplanted into the *M. konaensis* infested soil and grown for 25 weeks (Hurchanik and Schmitt, 2003; Hurchanik *et al.*, 2004).

Shoots of *Heterodera avenae* infected oat plants had significantly lower concentration of labelled potassium than that of healthy plants though there was no significant difference in the roots (Price *et al.*, 1982). Total uptake of potassium however relative to the size of the root system, was significantly greater in infested plants. Total uptake of phosphorus in shoots differed little between infected and uninfected plants but total uptake of phosphorus in roots was greater in the infested plants. Rao *et al.* (1988) reported that infestation of rice by rice root nematode, *Hirschmanella oryzae* and *M. graminicola* caused reduction in nitrogen, phosphorus and reducing sugars in shoots and roots. Total sugars, proteins, IAA, cytokinin and thiamine were reduced in the nematode infested plants and phenols were reduced in root knot nematode infested plants. Phenols decreased in shoots and increased in roots due to cyst nematode, while the root knot nematode caused similar changes in potassium. Infestation by both the nematodes caused nutritional disorders limiting the uptake of nitrogen and phosphorous and chlorosis of leaves.

Haseeb *et al.* (1990) found decrease in potassium concentration in roots and shoots of *Hyoscyamus niger* with *M. incognita* 90 days after inoculation. In case of soybean infected by *H. glycines*, the concentration of potassium (K) was decreased whereas root phosphorus (P) concentration was increased (Blevins *et al.*, 1995). The P concentration of cysts isolated from the soybean roots was high. Since a total uptake and root concentration of K was decreased, the infested soybean fields would require very high levels of potassium fertilization.

Bin Xu Ying *et al.* (1999) in pot experiments found that groundnut seeds from plants infected with *M. arenaria* showed reduced root absorbing activity and a lowered uptake of N, P, and K. Carneiro *et al.* (2002) showed that total amount of ^{15}N in the *M. incognita* infected soybean roots increased at the highest inoculum levels but ^{32}P decreased. In the shoots there was reduction of ^{32}P . The specific concentration of the labelled nutrients also showed a decrease of ^{32}P in the shoots and roots of infected plants and an increase of ^{15}N the shoots. The concentration and uptake of nitrogen, phosphorous and potassium was significantly reduced in cotton var. H 777 infected with *M. incognita* (Verma and Jain, 2006). Mahalik and Routaray (2009) investigated the changes of nitrogen content in superior mutant lines (M5) developed from three morphologically different black gram, *Vigna radiata* varieties (plant U-30, PDU-1 and Sarala) towards *M. incognita* infection. Nematode inoculation reduced nitrogen content in shoot of black gram by 11.41% respectively, but inoculated plant roots recorded 1.761% nitrogen which was 10.06% increase over the uninoculated plants. Sarala variety recorded higher nitrogen in shoot (3.05%) root (1.815%) as compared to other two parental varieties on these biochemical characters.

2.2. Effect of nematode on heavy metal uptake and translocation

In a study of tomato (*Lycopersicon esculentum* cv. Pusa Ruby) plants exposed to high soil Nickel (Ni) and nematodes, it was found that both interact to affect growth of young plants. Soils amended with Ni at 0, 10, 50, 100, 200 and 400 mg/L and subsequently inoculated with 2000 juveniles of *Meloidogyne incognita*/pot resulted in substantial growth reduction of tomato. Nickel amendments at 200 or 400 mg/L caused browning and/or necrosis of foliage. Either Ni or nematodes were able to decrease root and shoot growth, and carotenoid and chlorophyll contents of foliage. The order of Ni accumulation was: root > leaves > stem, and leaves > root > stem in infected and uninfected plants, respectively (Khan *et al.*, 2006).

Five doses of cobalt were tested for their action against the root-knot nematode, *Meloidogyne arenaria* infesting sugar beet and growth parameters, plant yield, sugar yield, chemical contents, mineral composition and total soluble solids (TSS%) under new reclaimed sandy loam soil. Gad and Ismail (2011) found that all cobalt doses significantly ($p \leq 0.05$ and / or 0.01) reduced juveniles, females, galls, egg-masses and eggs per egg-masses numbers as compared to un-treated plants. The rate of 7.5 ppm cobalt caused the maximum growth, root yield, mineral composition

and sugar yield of sugar beet compared to control and other cobalt rates. Cobalt at 7.5 ppm gave a superior result in chemical contents (%) as protein, carbohydrate, vitamin C, sucrose and glucose. Roots total acidity percentage decreased as cobalt addition increased and hence improved root quality.

Begum (2007) studied the effect of arsenic (As) on post infection development of root-knot nematode *M. incognita* in root of Roselle. The population of infective second stage larvae increased due to soil treatment with As at 40 and 80 ppm. After 80 ppm their populations decreased gradually with increased level of As. On the other hand, treatment of soil with As caused reduction in population of late second stage, third stage, fourth stage larvae, immature and egg laying females. The reduction was linear with the levels of As. In root tissues, infective second stage larvae of *M. incognita* developed into late second stage and third stage larvae within 3 and 12 days after inoculation, respectively. Fourth stage larvae, immature females and mature males appeared on 15th day, egg laying females appeared on 18th day of inoculation. The life cycle (larvae to egg) of the nematodes was completed in 18 days in roots of roselle growth in As treated and untreated soil. The results indicated that As did not affect duration on penetration after inoculation and transformation of infective larvae into different developmental stages. However, at 40 and 80 ppm of As rate of penetration was higher but at 120 ppm and above rate of penetration reduced as compared to control (0 ppm).

A field plot experiment to study the interactive effects of heavy metal (primarily nickel) and the root-knot nematode (*M. hapla*) on Celery. The treatment used were control soil (no metal or nematodes), non-metal soil plus nematodes (inoculated), heavy metal soil (nickel at 7500 ppm, copper at 800 ppm, and cobalt at 100 ppm) without nematodes, and heavy metal soil plus nematodes. Bisessar *et al.* (1983) found that the nematode treatment alone resulted in an average celery shoot weight 12% less than the controls, whereas heavy metals alone resulted in shoot weight 79% less than the controls. The combined effect of nematodes and heavy metals was a shoot weight 85% less than the controls. The roots of nematode-inoculated plants grown in heavy metal soil had significantly more nematode galls than did the roots of inoculated plants grown in non metal soil, indicating that heavy metals, primarily nickel, predisposed celery to greater attack by *M. hapla*.

2.2.1. Heavy metal uptake and translocation

Arsenic poisoning affects millions of people worldwide. Human arsenic intake from rice consumption can be substantial because rice is particularly efficient in assimilating arsenic from paddy soils, although the mechanism has not been elucidated. Ma *et al.* (2008) reported that two different types of transporters mediate transport of arsenite, the predominant form of arsenic in paddy soil, from the external medium to the xylem. Transporters belonging to the NIP subfamily of aquaporins in rice are permeable to arsenite but not to arsenate. Mutation in OsNIP2; 1 (Lsi1, a silicon influx transporter) significantly decreases arsenite uptake. Furthermore, in the rice mutants defective in the silicon efflux transporter Lsi2, arsenite transport to the xylem and accumulation in shoots and grain decreased greatly. Mutation in Lsi2 had a much greater impact on arsenic accumulation in shoots and grain in field-grown rice than Lsi1. Arsenite transport in rice roots therefore shares the same highly efficient pathway as silicon, which explains why rice is efficient in arsenic accumulation.

Xu *et al.* (2008) investigated the dynamics of As speciation in the soil solution under both flooded and aerobic conditions and compared As accumulation in rice shoot and grain in a greenhouse experiment. Flooding of soil led to a rapid mobilization of As, mainly as arsenite, in the soil solution. Arsenic concentrations in the soil solution were 7-16 and 4-13 times higher under the flooded than under the aerobic conditions in the control without As addition and in the +As treatments (10 mg As kg⁻¹ as arsenite or arsenate), respectively. Arsenate was the main As species in the aerobic soil. Arsenic accumulation in rice shoots and grain was markedly increased under flooded conditions; grain As concentrations were 10-15- fold higher in flooded than in aerobically grown rice. With increasing total As concentrations in grain, the proportion of inorganic As decreased, while that of dimethylarsinic acid (DMA) increased. The concentration of inorganic As was 2.6-2.9 fold higher in the grain from the flooded treatment than in that from the aerobic treatment. The results demonstrate that a greatly increased bioavailability of As under the flooded conditions is the main reason for an enhanced As accumulation by flooded rice, and growing rice aerobically can dramatically decrease the As transfer from soil to grain.

The objective of this study was carried out by Singh *et al.* (2011) to determine the accumulation and translocation of heavy metals in soil and in paddy crop irrigated

with lake water compared to soil and paddy crop irrigated with bore-well water. The quantities of heavy metals (Cd, Cr, Cu, Pb, Zn, As, Mn, and Hg) were determined in different parts of rice plants (*Oryza sativa*). Results revealed that the mean levels of soil Cd, Cr, Pb, Zn, As, Mn, and Hg in experimental soil and in different parts of rice plant (root, straw, and grain) were higher than the control except for Cu. The content of eight toxic metals was significantly higher in root than in aerial parts of the rice (straw and grains). Rice roots were enriched in Cd, As, Hg, and Pb from the soil, while Cr, Cu, Zn, and Mn were hardly taken by the roots. Bioaccumulation factor for Hg was significantly higher than other heavy metals. Metal transfer factors from soil to rice plants were significant for Cd, Cr, Cu, Pb, Zn, As, Mn, and Hg. The concentrations of metals in lake water were found to be within the permissible limit of Indian standard prescribed by Central Pollution Control Board (2000), except for Hg and As, which were higher than the limit of Indian standard. However, the concentrations of heavy metals in soil and rice grains were still below the maximal levels, as stipulated by Indian Prevention of Food Adulteration Act (PFA, 1954) and World Health Organization (WHO, 1993) guidelines.

Arsenate (As^{V}) is the predominant form of arsenic in soils under aerobic conditions and competes with the major plant nutrient phosphorus (P) in the form of phosphate (P^{V}) not only for sorption sites on mineral surfaces in soil but also for root membrane transporters. Plants have evolved several mechanisms for the mobilization of P^{V} in soils in response to P deficiency, such as the release of organic anions and protons. The study was to test whether these mechanisms result in a simultaneous mobilization of arsenate and what would be the consequences for As transfer from soil to plant. The compartment system approach with *Zea mays* as model crop was chosen as an experimental setup. The system is equipped with micro suction cups and allowed us to investigate processes occurring in the vicinity of roots. As a case study, an artificial quartz substrate with well defined soil physical properties was fertilized, spiked with As^{V} , and amended with increasing amounts of goethite (0, 1, and 4 g kg^{-1} in treatments G-0, G-1, and G-4, respectively). The addition of goethite alleviated the As^{V} -induced growth reduction and reduced As^{V} transfer from the substrate to the plant but induced P deficiency at the same time. When low amounts of goethite (1 g kg^{-1}) were added, plants mobilized P^{V} but not As^{V} , which might be related to differences in surface complexation reported for P^{V} and As^{V} . No mobilization of P^{V} or As^{V} was observed with the addition of higher amounts of

goethite, probably because of decreasing competition between organic anions, P^V , and As^V for binding sites (Vetterlein *et al.*, 2007).

The present study aimed to investigate the effects of root surface iron plaque on the uptake kinetics of arsenite and arsenate by excised roots of rice (*Oryza sativa*) seedlings. The results demonstrated that the presence of iron plaque enhanced arsenite and decreased arsenate uptake. Arsenite and arsenate uptake kinetics were adequately fitted by the Michaelis–Menten function in the absence of plaque, but produced poor fits to this function in the presence of plaque. Phosphate in the uptake solution did not have a significant effect on arsenite uptake irrespective of the presence of iron plaque; however phosphate had a significant effect on arsenate uptake. Without iron plaque, phosphate inhibited arsenate uptake. The presence of iron plaque diminished the effect of phosphate on arsenate uptake, possibly through a combined effect of arsenate desorption from iron plaque (Chen *et al.*, 2005).

2.3. Effect of heavy metals on seed quality of rice

Recent breakthroughs in rice arsenic (As) research demonstrate that As accumulation significantly affects trace nutrients in rice grain. Dwivedi *et al.* (2012) studied and analyzed the amino acid (AA) profile of sixteen rice genotypes differing in grain As accumulation, grown at three sites with different soil As concentrations, in ascending order, insurahb < Purbosthalib < Birnagar. Grain As accumulation negatively correlated with essential amino acids (EAAs) which were more prominent in high As accumulating rice genotypes (HAARGs). Conversely, non-essential amino acids (NEAAs) showed an increase in low As accumulating rice genotypes (LAARGs) but a decrease in HAARGs. EAAs like isoleucine, leucine, valine, phenylalanine, and tyrosine also decreased in most of the genotypes. NEAAs like glutamic acid, glycine, proline, and histidine showed an increase in all LAARGs. Likewise, sulphur containing AAs (methionine and cysteine) increased in LAARGs but decreased in HAARGs. Among NEAAs in HAARGs, only arginine and serine showed some induction in most of the genotypes. At the highest As site (Birnagar) total EAAs and NEAAs show significant reduction in HAARGs compared to LAARGs. The study concluded that As accumulation in rice grain alters EAAs and NEAAs differentially, and reduction was more pronounced in HAARGs than in LAARGs. Thus, As tainted rice limits required levels of AAs in rice based diets and therefore cannot alone fulfil the recommended daily intake (RDI) of AAs.

Ji *et al.* (2012) conducted a field survey to evaluate the criteria of cadmium pollution in paddy soil. A set of 32 samples of paired grain, straw, and topsoil (0-20) were collected, and Cd concentrations in soils and rice plants were determined by an ICP-MS. The results indicated that Cd concentrations of fifteen soil samples were excessive to the value of maximum allowable limit (MAL) for cadmium in soil (1.0 mg kg⁻¹), and Cd concentrations of five brown rice samples were excessive to maximum level for cadmium in rice (0.2 mg kg⁻¹ dry matter). It was found that Cd concentrations in straw and brown rice are significantly ($P < 0.01$) correlative to the Cd concentrations in topsoil with the correlative coefficients 0.800** and 0.907**, respectively. Using the regression equation and maximum level for Cadmium in rice, the criteria of Cd pollution in paddy soil modified as 1.5 mg kg⁻¹ was recommended. Cadmium concentration in straw could be used for early distinguishing the status of cadmium contamination of rice grain.

The following table describes the interaction between various microorganisms/plant parasitic pathogens and heavy metals/nutrients and how they play a role in uptake/translocation of these elements in to plants from soil to roots, shoot and grains.

Heavy metals/ nutrients	Crops	Plant parasitic nematodes/ pathogens	Interaction effects/causes	Reference
Bacteria and heavy metals interaction				
Cadmium (Cd) and Zinc (Zn)	<i>Sedum alfredii</i>	Bacteria (<i>Burkholderia cepacia</i>)	Increased plant growth, P, and metal uptake in shoots, better translocation of metals from root to shoot.	Li <i>et al.</i> , 2007
Zn, Cd and Lead (Pb)	Willows (<i>Salix caprea</i>)	<i>Pseudomonas</i> spp. <i>Janthinobacterium</i> sp. <i>Serratia</i> sp. <i>Flavobacterium</i> sp. <i>Streptomyces</i> sp. and <i>Agromyces</i> sp.	Decreased metal mobilization and plant growth.	Kuffner <i>et al.</i> , 2008
Zn	<i>Orychophragmus violaceus</i>	Zn-tolerant bacterial strains, <i>Bacillus subtilis</i> , <i>B. cereus</i> , <i>Flavobacterium</i> sp. and <i>Pseudomonas aeruginosa</i>	Increased root elongation and shoot biomass and Zn accumulation in <i>O. violaceus</i> . Increased availability of water-soluble Zn in soil and Zn accumulation by plants.	He <i>et al.</i> , 2010

Ni	<i>Pseudomonas</i> spp.	Canola (<i>Brassica napus</i> L.)	Increased about 60% in <i>P. fluorescens</i> inoculated canola over uninoculated plants under high Ni level (2 mM).	Ashour <i>et al.</i> , 2006
Fungi and Heavy metals interaction				
Cd, Zn, Cu, Pb, Manganese (Mn)	Maize (<i>Zea mays</i> L.)	Arbuscular mycorrhizal fungus, <i>Glomus mosseae</i>	Fourfold higher shoot and tenfold higher root biomass. Decreased Cd, Cu, Zn and Mn concentrations.	Weissenhorn <i>et al.</i> , 1995
Phosphorus (P) and Arsenic (As)	Lentil (<i>Lens culinaris</i> L.)	Arbuscular mycorrhizal fungus, <i>Glomus mosseae</i>	Increased “P” uptake and enhanced “P” nutrition. Decreased uptake of toxic As into plant parts.	Fazel <i>et al.</i> , 2011
Plant virus and heavy metals interaction				
Cu, Zn, Pb, Cd and As	Tomato (<i>Solanum lycopersicum</i>)	Cucumber mosaic virus (CMV) and Tomato mosaic virus (ToMV)	Decreased the accumulation rate of heavy metals such as Cu, Zn, Pb, Cd and As in tomatoes.	Miteva <i>et al.</i> , 2001

Plant parasitic nematodes and heavy metals interaction				
Cobalt (Co)	Sugar beet, <i>Beta vulgaris</i> L.	Root-knot nematode, <i>Meloidogyne arenaria</i>	Increased the growth, and yield of roots, as well as sugar yield and root mineral composition (except Fe content) protein, carbohydrate, vitamin "C", sucrose and glucose.	Gad and Ismail, 2011
Nickel (Ni)	Tomato (<i>Solanum lycopersicum</i> cv. Pusa Ruby)	Root-knot nematode, <i>Meloidogyne incognita</i>	Nickel amendments at 200 or 400 mg/L caused browning and/or necrosis of foliage. Increased Nematode disease severity (i.e., degree of galling) when plants received a Ni amendment of 50 or 100 mg/L. Nickel contents of roots, stems and foliage were greater in nematode infected plants than uninfected plants grown in soils receiving no Ni amendments. The order of Ni accumulation was: root > leaves > stem, and leaves > root > stem in infected and uninfected plants, respectively	Khan <i>et al.</i> , 2006
Ni	Celery (<i>Apium graveolens</i>)	Root-knot nematode, <i>Meloidogyne hapla</i>	Growth reduction	Bisessar <i>et al.</i> , 1983

Plants and heavy metals/nutrients interaction

As	Wheat (<i>Triticum aestivum</i> L.)	<p>Increased seed germination and growth of root and shoot at low concentrations of As (0–1 mg/kg), however, these factors all decreased gradually at high concentrations of As (5–20 mg/kg).</p> <p>The contents of O₂⁻, MDA, soluble protein and peroxidase (POD) activity all increased with increasing As concentrations.</p> <p>Soluble sugar content, Ascorbate peroxidase (APX), and Superoxide dismutase (SOD) activities decreased at low concentrations of As, and increased at high concentrations of As.</p> <p>While acetylsalicylic acid (ASA) and chlorophyll contents, catalase (CAT) activity displayed increasing trend when the concentrations of As was lower than 1 mg/kg, and then decreasing trend.</p>	Xi <i>et al.</i> , 2006
----	--------------------------------------	--	-------------------------

As	Wheat (<i>Triticum aestivum</i> L.)	<p>Decreased root, stem and spike dry weight and grain yield per plant.</p> <p>Arsenic concentrations in plant tissues were as follows: roots > stems > leaves and rachises > grains> glumes > awns.</p> <p>Arsenic concentrations in bran were about 2-3 times higher than those in flour.</p> <p>Most of the arsenic contaminated flour exceeded the Chinese tolerance limit.</p>	Zhang <i>et al.</i> , 2009
As	Onion(<i>Allium cepa</i>)	<p>Increased chlorophyll a and b contents in onion leaf.</p> <p>The highest chlorophyll-a (0.004847/g) and chlorophyll-b (0.006528/g) contents were estimated in the onion leaf irrigated with 0.800mg/l of Arsenic whereas, in control plant it was lowest (chl-a 0.002363/ and chl-b 0.004092/g).</p>	Sushant and Ghosh, 2010

As	Castor Bean (<i>Ricinus communis</i>)	Free inorganic As, mainly As(III) was transported in the phloem of castor bean exposed to either As(V) or As(III), and that methylated As species were more mobile than inorganic As in the phloem.	Ye <i>et al.</i> , 2010
----	---	---	-------------------------

Investigations on the effect of root-knot nematode infection on the uptake of nutrients and heavy metals by rice plants included experiments in laboratory and field. General materials and methods are described briefly in this chapter, while the specific techniques are explained in the relevant research papers in the next chapter.

3.1 Collection and maintenance of root knot nematode, *Meloidogyne graminicola*

In the month of May 2010 soil was collected from the root-knot nematode infested rice field of the IARI research farm, New Delhi. Rice, *Oryza sativa* cv. Pusa 1121 seed was sown in the collected soil in 25 cm diameter clay pots. Galls found on the roots of 30 day old rice seedlings were collected. *Meloidogyne* females were dissected out of these galls. An examination of the perineal pattern having tail whorl and ESPT ratio of 2.5 (Jakson, 1977) confirmed species as *Meloidogyne graminicola* (Kofoid and White, 1919; Golden and Birschfield, 1965). The infected rice seedlings were collected and pure culture was maintained in a glasshouse at $27 \pm 2^\circ$ C for further study.

3.2 Stock culture and maintenance

The galls with egg masses inside the root cortex were dissected and incubated at $27 \pm 2^\circ$ C for 48 h to obtain freshly hatched second stage juveniles (J_2). These were used to establish stock culture for use in experiments on effect of *M. graminicola* on plant growth, nutrient and heavy metal uptake. *M. graminicola* was maintained in rice. Three week old young rice cv. Pusa Basmati 1121 plants were inoculated with chopped infected root material and hatched larvae. Plants were watered as required (depending on the moisture content of the top of the soil in the pots) with tap water to maintain the soil in a moist state and maintained in glasshouse at $27 \pm 2^\circ$ C.

3.3 Nematode extraction and inoculation

Rice root galls with embedded egg masses were collected from infected rice plants after removing the shoots and carefully washing the roots in cold tap water. Using an adaptation of Whitehead and Hemming's (1965) method, galls with embedded egg masses were placed onto 4 cm diameter 90 mesh nylon sieves on glass

Petri dishes filled with distilled water. In order to improve aeration, level of water in Petri dishes was adjusted so as to partly cover the egg masses. The required numbers of Petri dish/sieve extraction units were placed in trays, covered with aluminium foil and eggs hatched at $27 \pm 2^\circ \text{C}$ in a BOD incubator or at room temperature in the laboratory.

3.4 Measurement of nematode infection

The level of *M. gramnicola* infection on rice root was measured by assigning a galling index (0-5) to the root systems (Taylor and Sasser, 1978) based on the approximate proportion of root showing simple and multiple galls.

3.5 Estimation of nutrients and heavy metals in roots, shoots and grains

At mid-season of 45 days after transplanting (DAT) and final stage (110 DAT) of the rice plants, matured roots, shoots and grains (grains were collected at the time of harvesting) were collected for nutrient and heavy metals analysis from randomly chosen plants from each pot and selected field spots. Plant samples were oven dried at 65°C , and powdered in pestle and mortar. Total nitrogen from plant was estimated in 1g sample, subjected to H_2SO_4 digestion with the help of Kjeldhal automatic digester by Kjeldhal method. In diluted samples 15ml of water was added then samples were distilled with NaOH and the finally titrated with hydrochloric acid (HCL) (Jackson, 1973).

The phosphorus and potassium were determined in 1g dried plant samples. Plant samples were subjected to nitric-perchloric acids (3:1, v/v) digestion. Digested samples were used for analysis of phosphorus by Spectrophotometer (model UV 1800) using vanadate-molybdate reagent (Olsen *et al.*, 1954). Available potassium was determined by using Flame Photometer (model ME 881). Heavy metals, As, Pb and Cd concentration in the plants were determined by Atomic Absorption Spectrophotometer (model UV 1800).

3.6 Plant growth parameters

The plant height (cm) was measured with help of a scale of ten randomly selected plants at the time of maturity. The plants after harvesting were cut into shoot and root portion separately. Shoot and root portions were washed thoroughly with water for removing soil particles and other materials and kept in dry oven for 24 hr at

65°C. Shoot and root portions were washed thoroughly with water for removing soil particles and other materials and kept in hot air oven at 65°C for 72 hr and their dry weights (g) were recorded using electronic weighing machine. Number of galls on roots per plant root system was also counted.

3.7 Statistical analysis

The data were subjected to factorial analysis of variance (ANOVA) in CRD and RBD, as applicable. The differences between means were tested for significance at $P=0.05$ by using OPSTAT statistical software.

3.8 Experimentation

The following experiments were conducted at IARI research farm, New Delhi:

1. To study the effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of macro-nutrients, NPK and plant growth of rice.
2. To study the effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of heavy metals, Arsenic, Cadmium and Lead and growth of rice.
3. To find out whether nematode infection can influence the health hazard due to heavy metal uptake by rice cultivated in naturally heavy metal rich or polluted soils.

Effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of macronutrients and Arsenic and plant growth of rice

Abstract

Root-knot nematodes are known to induce morphological and physiological changes in tissues that affect the ability of the root to take up and transport water and mineral nutrients from the soil. Accumulation of toxic heavy metals in soil and possibility of their uptake by plants is also an emerging concern with increasing soil pollution. Investigations have been carried out on the effect of the root-knot nematode, *Meloidogyne graminicola*, a serious pest of rice, on the content of the major plant nutrients, viz. Nitrogen (N), Phosphorus (P) and Potassium (K) and the heavy metal, Arsenic (As) and growth of the *basmati* rice, *Oryza sativa*, cv. Pusa 1121. The content of NPK after 45 and 110 days of transplanting was significantly lower in the shoot of rice inoculated with 2 and 8 J2 cm⁻³ soil compared to nematode-free plants, the reduction being more at the higher inoculum level. To the contrary, content of As in the plants grown in soil artificially contaminated with 5 and 10 ppm As increased upto three times due to the nematode infection of roots, the increase being more at the higher inoculum level. The As content in plant was more at the higher soil contamination level. The plant growth in terms of height and dry weight significantly (0.05 P) decreased upto 50% and negatively correlated with the nematode inoculum level. In general, As contamination of soil upto 10 ppm did not cause significant reduction in plant growth or root galling due to *M. graminicola* but the trends indicated possibility of toxicity to both plant and nematode at higher As contamination levels. Similar effects were observed in artificially contaminated sandy-loam of Delhi and silty-clay loam of West Bengal. No As was detected in the shoot or grain of rice plants grown in untreated sandy loam soil of IARI farm, Delhi used in these experiments without or with *M. graminicola* inoculation. The results confirmed that *M. graminicola* infection of root can not only reduce uptake of NPK and decrease plant growth but it can also increase health hazard for humans and other consumers due to the increased uptake of the toxic heavy metal, Arsenic.

Key words: Nitrogen, Phosphorus, Potassium, Arsenic, Heavy metal, Soil pollution, *Meloidogyne graminicola*, Nematode, Rice.

Introduction

The root-knot nematode, *Meloidogyne graminicola*, has emerged as a serious pest of rice in most rice growing regions of the world (Gaur & Pankaj, 2011). In India the nematodes are known to cause damage especially in rice nurseries and upland rice crops in the eastern and southern coastal region but are now prevalent in most rice growing regions of the country. In the north Indian Indo-Gangetic plains, Gaur *et al.* (1993) reported two species of root-knot nematodes, viz. *M. graminicola* and *M. triticooryzae* infesting both wheat and rice crops grown in succession in the rice-wheat cropping systems. Rice is not only a staple food but also commercially important in trade; especially the scented basmati rice which is a premium product having high market demand in India as well as for export. Therefore, quantity of produce as well as quality is important factors determining production and nutritional and commercial value.

It is well known that the symptoms of nematode damage appear as chlorosis and stunted growth, poor tillering, inflorescence and grain production (Swain and Prasad, 1988). It has been demonstrated that these symptoms are the result of reduced uptake of water and nutrients by the plant roots structurally modified and damaged by the nematode (Patnaik and Padhi, 1987). Histological studies have shown disruption of the xylem tissues in galls as well as the presence of abnormally formed xylem vessels causing interruption of induced root water transport (Kirkpatrick *et al.*, 1991) and consequently the translocation of absorbed nutrients to the shoot. Patil *et al.* (2013) have demonstrated reduction in uptake of macronutrients, Nitrogen (N), Phosphorus (P), Potassium (K) and micronutrients, Iron (Fe) and Zinc (Zn) due to *M. graminicola* infection of the roots.

Heavy metal contamination of soils and their uptake by plants especially, the increase in their concentration in the consumable parts like straw or grain has become serious concern because of the possible health hazards to animals and including live stock and human beings (Sanyal *et al.*, 2012). The heavy metal, Arsenic (As) is one of the most toxic elements even at low concentration. It is known to accumulate more in low oxygen (O₂) condition typical of the soil conditions in heavy submerged soils. Normally As occurs in soils in the less toxic arsenate and more toxic arsenite forms,

the latter accumulating more under low oxygen ecologies. Food is a potentially important source of dietary As intake (Vahter *et al.*, 1995, Schoof *et al.*, 1999, Das *et al.*, 2004). Rice (*Oryza sativa*) accumulates the highest amount of As of all grain crops, because of the anaerobic conditions prevailing in paddy soil, which lead to arsenite mobilization (Takahasi *et al.*, 2004; Williams *et al.*, 2007 and xu *et al.*, 2008, Wang *et al.*, 2010; Zheng *et al.*, 2013) and the inadvertent uptake of arsenite through the rice silicic acid uptake pathway (Ma *et al.*, 2008). Rice is one of the major staple food crops in the world, with daily intake up to 0.5 kg (dry weight) per capita in some Asian countries (Anon, 2001). Consequently, rice is a potentially major source of dietary As for much of the world's population, with the fraction that is inorganic As varying from 10 to 90% (Williams *et al.*, 2005). Reported levels of As in rice are <0.01–2.05 mg kg⁻¹ for Bangladesh, 0.31–0.70 mg kg⁻¹ for China, 0.03–0.044 mg kg⁻¹ for India, 0.08–0.38 mg kg⁻¹ for Italy and Spain, <0.10–0.76 mg kg⁻¹ for Taiwan, 0.11–0.66 mg kg⁻¹ for the USA and 0.03–0.47 mg kg⁻¹ for Vietnam(Williams *et al.*, 2005; Duxbury *et al.*, 2003; Caroli *et al.*, 2007; Caroli *et al.*, 2002; Pizarro and Gómez, 2003).

No reliable information is available on the effect of nematodes on uptake of As by plants. The present studies have been carried out on the effect of *M. graminicola* on the uptake of N, P, and K and As and plant growth of rice, to understand whether the uptake of As would follow same trend as reduced uptake of micro- and macronutrients due to increased nematode infestation levels or not. It would be relevant to know if there would be some indirect benefit in terms of reduced As risk due to possible reduced uptake in response to nematode infection.

Materials and Methods

A preliminary study was done to see the effect of *M. graminicola* inoculation @ 4 J₂ cc⁻¹ on the As content of root and shoot of Rice cv. Pusa 1121 grown in clay loam soil containing 12 and 20 ppm As, obtained from BCKV, Kalyani. The 25 g soil with 12 and 20 ppm As contamination levels was filled in 5 cm diameter cups each. Six cups at each level were inoculated with *M. graminicola* and rice seed was sown. Each treatment was replicated three times. After 20 days of sowing the rice seedlings were uprooted and As content was determined in the root and shoot separately, using techniques mentioned in a following paragraph.

The effects of two inoculum levels of *M. graminicola* on the uptake of macronutrients, Nitrogen (N), Phosphorous (P) and Potassium (K) and heavy metal, Arsenic (As) and plant growth of rice were studied under pot culture. Seed of susceptible rice cv. Pusa 1121 was sown in steam sterilized soil in 25 cm diameter earthen pots. One month old seedlings were transplanted, four each in 25 cm diameter plastic pots containing about 3000 cm³ autoclaved soil and sand mixture (3:1). One week after transplanting, the seedlings were inoculated with nematodes @ 0, 2, 8 J₂ cm⁻³ of soil. All treatments were replicated four times and the pots were placed randomly in polygreenhouse at 27± 2°C. Uninoculated plants served as control. The pots were irrigated with deionised water and soil was kept near saturation.

The recommended dose of NPK was applied in soil before transplanting of rice. For heavy metal, Sodium arsenate dibasic heptahydrate (Na₂HAsO₄ · 7 H₂O), salts were added to attain As (0 ppm, 5 ppm and 10 ppm), one month prior to transplanting of rice. For analysis of nutrients in the plant samples, total nitrogen content in plant was estimated in 1g dried samples by Kjeldhal method (Jackson, 1973). For phosphorus and potassium analysis, 1g dried plant samples were digested with nitric and perchloric acids. Phosphorous was assessed by Spectrophotometer (UV 1800) using vanadate-molybdate reagent (Olsen *et al.*, 1954). Available potassium was determined by using Flame Photometer (ME 881). Heavy metal, As content in the plant samples were determined by Atomic Absorption Spectrophotometer (UV 1800).

The growth of plants was recorded 110 days after transplanting (DAT) in pot experiments. Height of ten randomly selected plants was measured at the time of maturity. After carefully harvesting, the plants were cut into shoot and root portion separately for determining dry weights. Shoot and root portions were washed thoroughly with water for removing soil particles and other materials and kept in dry oven at 65°C for 72 h. Dry weights were recorded using an electronic balance. Number of galls on roots per plant root system was also counted and used to grade root-knot index on a 0-5 scale.

Results

The data obtained in the preliminary experiment on the As contaminated soil from West Bengal indicated higher contents of As in the root and shoot of 20 day rice

seedlings grown in soil infested with *M. graminicola* @ 4 J₂ cm⁻³ than the nematode-free soil. (Fig. 4.1.1). Also higher contamination level of 20 ppm resulted in higher levels of As in both root and shoot.

The N content in the shoot of rice grown in nematode free soil without added As (control) was 0.47%. There was no significant change due to 5-10 ppm As to soil. A slight reduction in N content was observed in plants at *M. graminicola* inoculation levels of 8 J₂ cm⁻³ soil but the differences were not statistically significant at 0.05P. The main effect of As on the N content was not significant (Table. 4.1.1, Fig. 4.1.2). To the contrary, the N content was drastically reduced due to *M. graminicola* inoculation at 2 and 8 J₂ cm⁻³ soil with or without As treatment. Averaged over all the As levels, the N content was decreased from 0.46% to 0.38 (-17.4%) and 0.32 (-30.4%) respectively, at 2 and 8 J₂ cm⁻³ soil. Thus, the main effect of *M. graminicola* inoculation in reducing N content of shoot was significant (0.05 P). However, the interaction effect of the nematode and As was not significant statistically.

Similar trend was observed in the case of Phosphorus and Potassium contents of the shoot of rice, with minor variations. The main effect of *M. graminicola* inoculation decreased the P content from 0.18 to 0.10 (-44.4 %) and 0.09 (-50.0%) at the 2 and 8 J₂ cm⁻³ soil, respectively. There was no reduction in the P content at 0 and 2 J₂ cm⁻³ soil but at 8 J₂ cm⁻³, it was 0.11% in the absence of added As and reduced to 0.06% (-45.5%). The main effect of As and its interaction with the nematode however, remained non-significant (0.05P). Similarly, the main effect of *M. graminicola* in reducing the K content was significant. The average K content was 1.31% in the shoot of healthy plants; it was reduced greatly due to *M. graminicola* but only slightly due to the added As to the soil. Averaged over the As levels, the K content was decreased from 1.24% in healthy plants to 0.96 (-22.6%) and 0.80% (-35.5%) at the 2 and 8 J₂ cm⁻³ soil, respectively (Table 4.1.1, Fig. 4.1.2). The main effect of As as well as the interaction with nematode were non-significant statistically, however, it could be seen that averaged over the nematode inoculation levels, the K content was reduced from 1.10% to 1.0 (-9.1%) and 0.90 (-18.2%), respectively at the 5 and 10 ppm added As levels.

Since the focus of these investigations was more on Arsenic uptake, its content was studied in root and shoot at 45 and 110 DAT. No As was detected in the root or shoot of rice cv. Pusa 1121 plants grown in sandy loam soil collected from IARI farm, New Delhi. The treatment of this soil @ 5 and 10 ppm resulted in 0.43

Table. 4.1.1. Effect of Arsenic and root-knot nematode, *Meloidogyne graminicola* on the macro nutrients, NPK in shoot of rice cv.Pusa 1121.

Nematode inoculum level (J ₂ /cm ³ soil)	Nitrogen (%)				Phosphorus (%)				Potassium (%)				
	Arsenic (ppm)			Mean N	Arsenic (ppm)			Mean N	Arsenic (ppm)			Mean N	
	0	5	10		0	5	10		0	5	10		
0	0.47	0.46	0.45	0.46	0.19	0.18	0.18	0.18	1.31	1.33	1.08	1.24	
2	0.40	0.39	0.34	0.38	0.10	0.10	0.11	0.10	1.16	0.95	0.77	0.96	
8	0.31	0.36	0.28	0.32	0.11	0.12	0.06	0.09	0.85	0.72	0.85	0.80	
Mean A	0.39	0.40	0.36		0.13	0.13	0.11		1.10	1.00	0.90		
Nematode (N) CD 0.05 P				0.04					0.02				
Arsenic (A) CD 0.05 P				NS					NS				
N X A CD 0.05 P				NS					NS				

Table. 4.1.2. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Arsenic uptake by rice cv. Pusa 1121

Arsenic uptake (ppm) (Initial observation, 45 Days After Transplanting)									
Nematode inoculum level (J₂/cm³ soil)	ROOT				SHOOT				
	0	5	10	Mean N	0	5	10	Mean N	
0	0.00	0.43	0.82	0.41	0.00	0.36	0.32	0.23	
2	0.00	1.10	2.09	1.06	0.00	0.83	1.20	0.70	
8	0.00	1.99	3.13	1.70	0.00	1.70	1.90	1.20	
Mean A	0.00	1.17	2.01		0.00	0.96	1.16		
Nematode (N) CD 0.05 P				0.40					0.38
Arsenic (A) CD 0.05 P				0.40					0.38
N X A CD 0.05 P				0.70					0.67

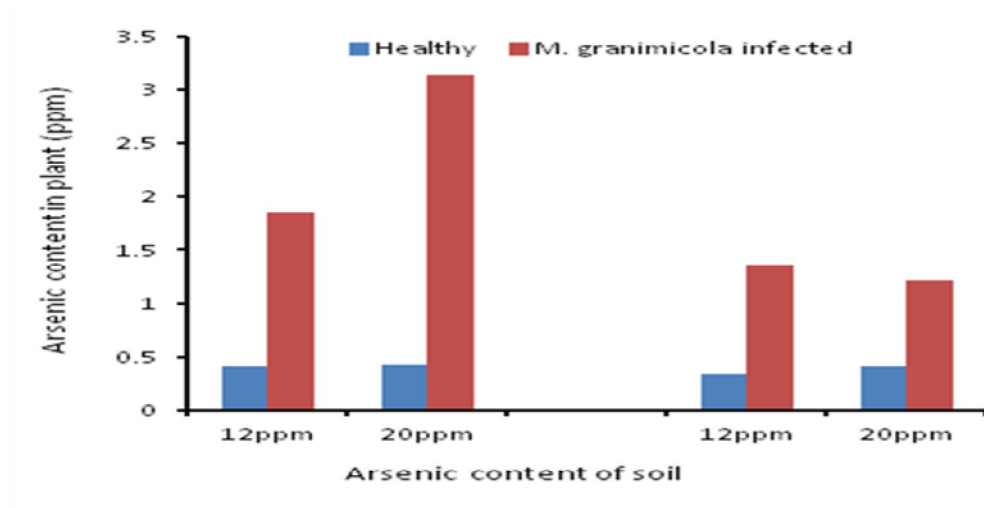


Fig. 4.1.1. Effect of *Meloidogyne graminicola* @ 4 J2 cm⁻³ on Arsenic content in root and shoot of 20 day rice plants grown in soil from West Bengal artificially contaminated Arsenic.

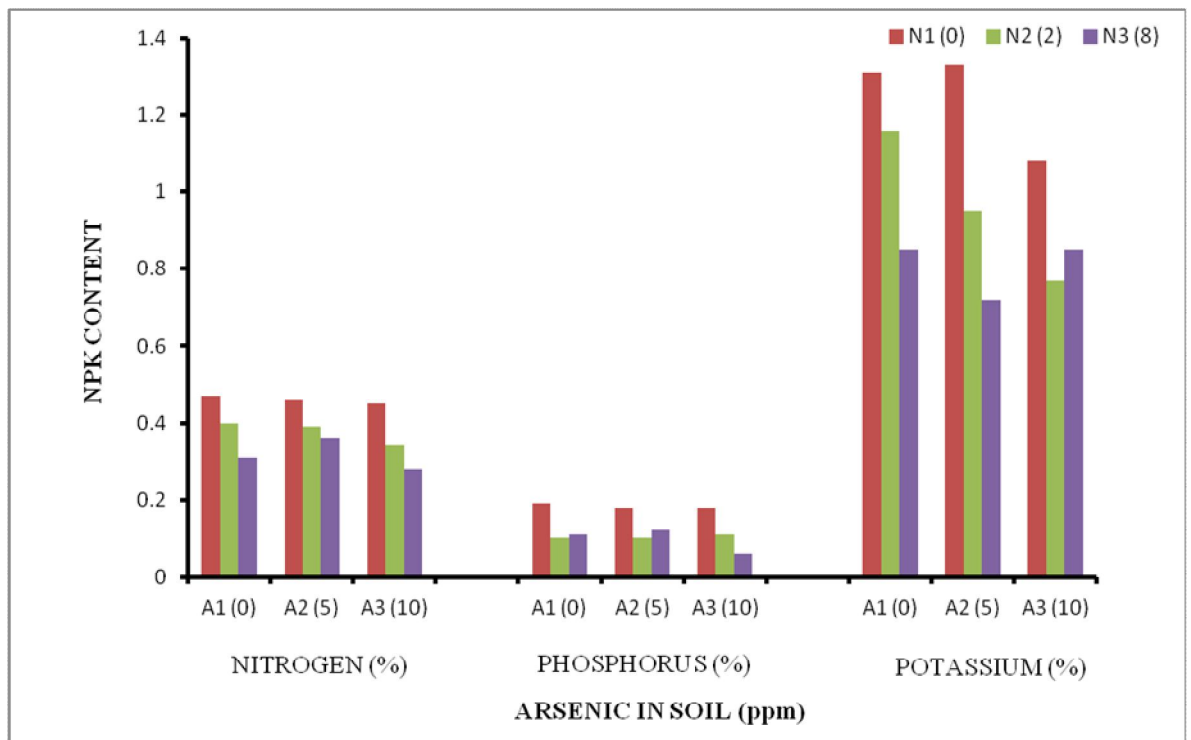


Fig. 4.1.2. Effect of Arsenic and root-knot nematode, *Meloidogyne graminicola* on the macro nutrients, NPK in shoot of rice cv. Pusa 1121. (A- Arsenic, N- Nematode)

and 0.82 ppm As in root and 0.36 and 0.32 ppm in shoot, respectively, in the case of nematode free plants. In the case of *M. graminicola* 2 J₂ cm⁻³ soil inoculation, these levels increased to 1.10 and 2.09 ppm in root and 0.83 and 1.20 ppm in shoot, respectively. The increase in *M. graminicola* inoculation levels to 8 J₂ cm⁻³ soil resulted in further increased in As content, both in root and shoot (Table. 4.1.2, Fig. 4.1.3). The main as well as interaction effects of *M. graminicola* and As treatment levels were significant at 0.05P.

The main effect of *M. graminicola* inoculation at 2 and 8 J₂ cm⁻³ soil resulted in 1.59 and 3.15 times increase in As content in root, 2.04 and 4.22 times increase in shoot. In general, the As content was higher in root than in shoot. The increases in As content in both root and shoot were not in exact direct proportion of the increase in level of As contamination of soil or the inoculum level of *M. graminicola*. Increase in added As from 5 to 10 ppm resulted in 71.8% and 20.8% increase in As content of root and shoot, respectively, averaged over the *M. graminicola* inoculum levels. Similarly, the increase in inoculum level from 2 to 8 J₂ cm⁻³ soil resulted in 60.4 and 71.8% increase in As content of root and shoot, respectively. The interaction effect would show that proportionate increase in As contents of root and shoot were lesser at higher *M. graminicola* inoculum levels, although the As content was definitely higher than the lower nematode inoculum and As treatment level.

The observation at 110 DAT revealed that the As content in both root and shoot increased with age of plants, thus indicating As accumulation in plant tissues (Table 4.1.3 Figure 4.1.4). More or less similar trends were observed at 110 DAT as at 45 DAT; with minor variations in proportionate increased in relation to As concentration and nematode inoculum levels. The increase in As content in grain was apparent. No As was detected in the grain harvested from plants grown in soil without As treatment. At 5 ppm As contamination treatment of soil, 0.44 ppm As was detected in the grain of nematode free plants; it increased to 0.60 and 1.03 ppm at 2 and 8 J₂ cm⁻³ *M. graminicola* inoculation levels. Similar trends were noticed at 10 ppm As contamination treatments. At 10 ppm As contamination treatment of soil and 8 J₂ cm⁻³ *M. graminicola* inoculum, the As content in the grain was alarmingly high at 2.18 ppm. The effect of *M. graminicola* inoculum was apparent in the reduced plant growth of rice measured in terms of shoot height and dry weights of root and shoot.

Table. 4.1.3. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Arsenic uptake by rice cv. Pusa 1121

Arsenic uptake (ppm) 110 DAT													
Nematode inoculum level (J₂/cm³ soil)	Root				Shoot				Grain				
	0	5	10	Mean N	0	5	10	Mean N	0	5	10	Mean N	
0	0.00	0.40	1.18	0.53	0.00	0.30	0.82	0.37	0.00	0.44	0.26	0.23	
2	0.00	1.43	2.83	1.42	0.00	1.18	1.87	1.02	0.00	0.60	1.01	0.53	
8	0.00	1.68	4.21	1.96	0.00	2.40	2.90	1.77	0.00	1.03	2.18	1.07	
Mean A	0.00	1.17	2.74		0.00	1.29	1.86		0.00	0.69	1.15		
Nematode (N) CD 0.05 P				0.44					0.56				
Arsenic (A) CD 0.05 P				0.44					0.56				
N X A CD 0.05 P				0.77					0.97				

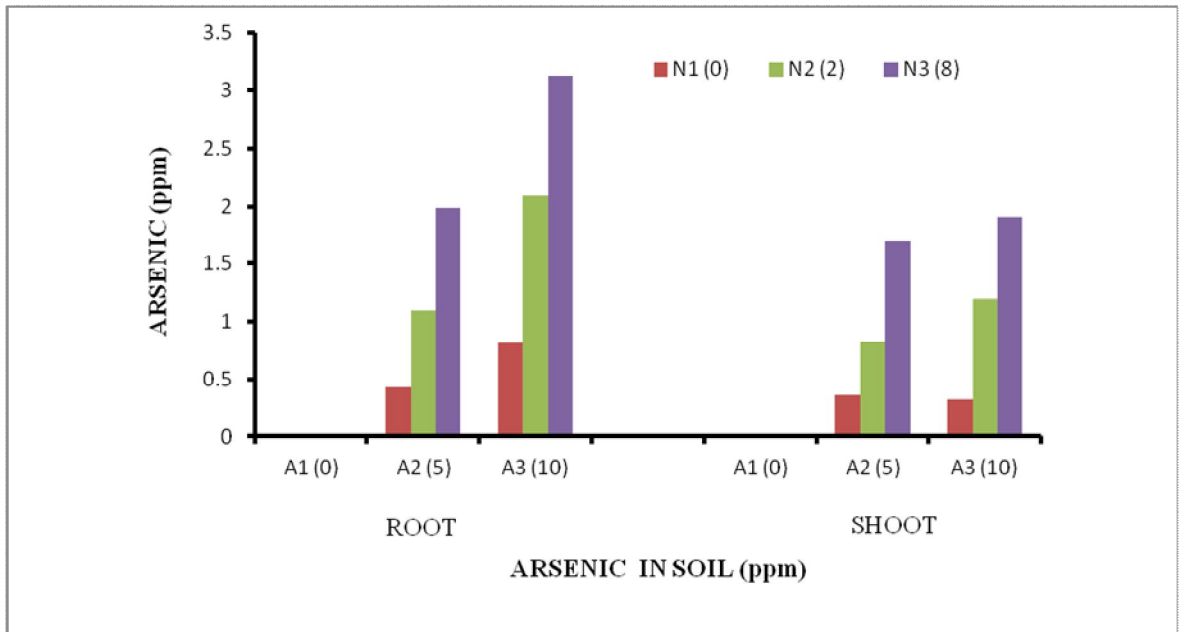


Fig. 4.1.3. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Arsenic uptake by rice cv. Pusa 1121, 45 days after transplanting. (A-Arsenic, N-Nematode)

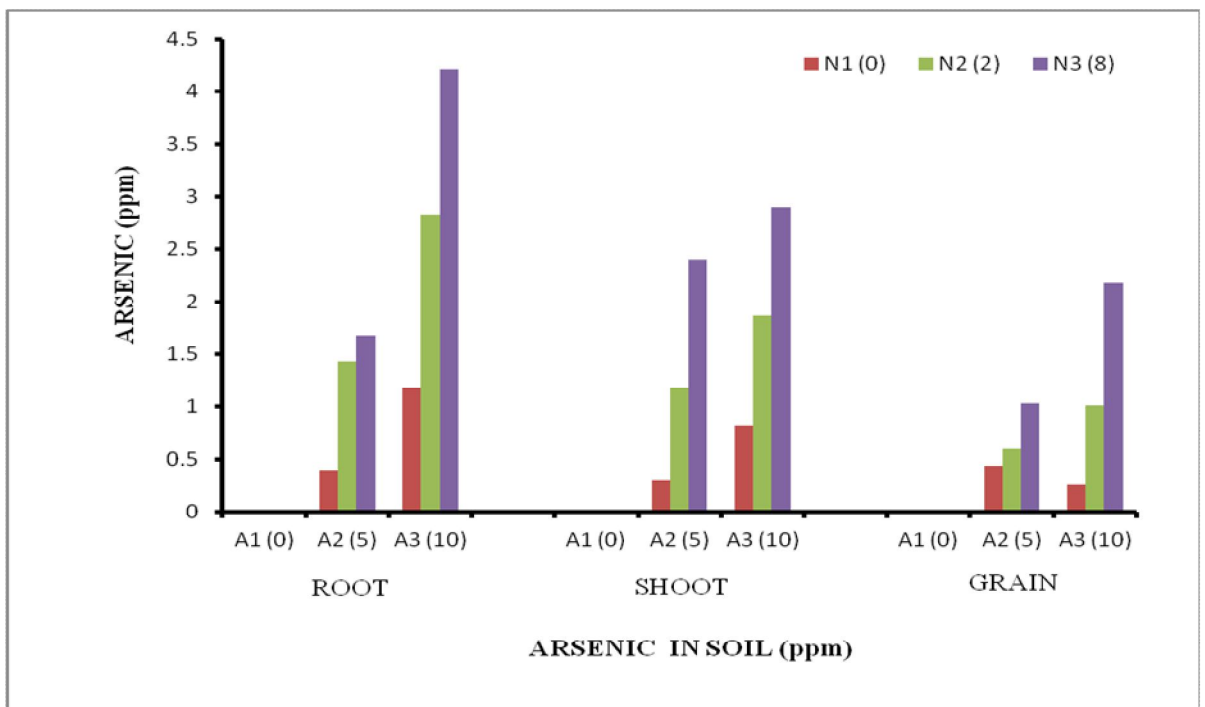


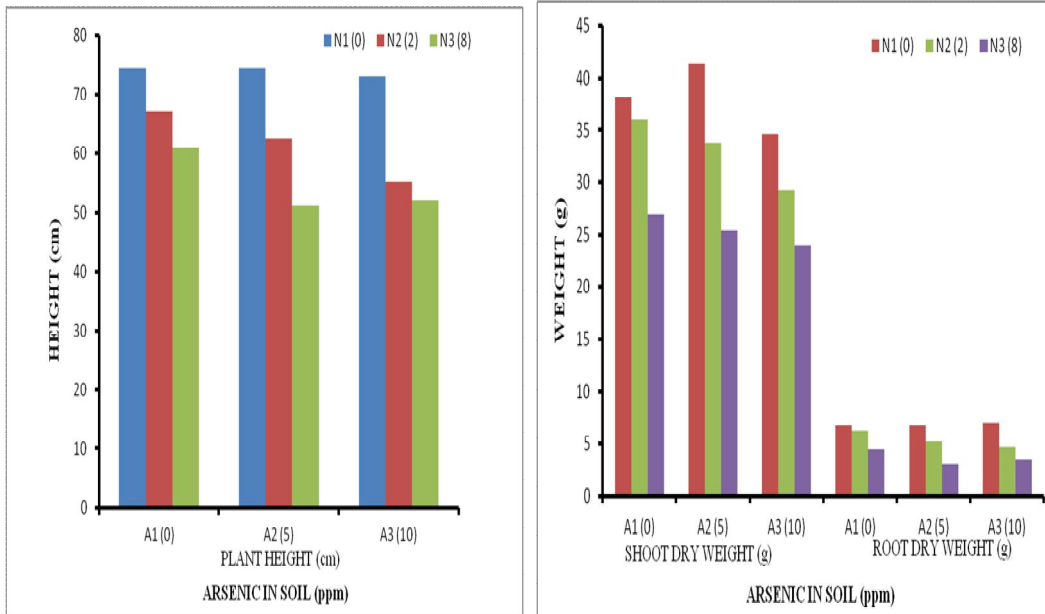
Fig. 4.1.4. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Arsenic uptake by rice cv. Pusa 1121, 110 days after transplanting. (A-Arsenic, N-Nematode)

The root dry weight was decreased by 22.4 and 46.8%; shoot height by 16.6 and 25.9% and shoot dry weight by 13.2 and 33.1%, respectively, at *M. graminicola* inoculum levels of 2 and 8 J₂ cm⁻³ soil (Table. 4.1.4, Fig. 4.1.5). It is interesting to note that although the main effect of As on the NPK content of shoot was non-significant, there was significant (0.05 P) reduction in root dry weight (14.6 and 14.3%), plant height (7.0 and 10.9%) and shoot dry weight (0.6 and 12.9%) at 5 and 10 ppm contamination treatments of soil, respectively. The interaction effect of As and *M. graminicola* on plant growth characters was not significant at 0.05 P. The number of galls on the root system was significantly higher though not in the same ratio at the inoculum level of 8 J₂ cm⁻³ compared to the lower levels of 2 J₂ cm⁻³ soil. However, the As contamination of the soil at 5 or 10 ppm did not cause reduction in root galling.

Table. 4.1.4. Effect of Arsenic and root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 1121

Plant growth parameters, 110 DAT (Arsenic treated soil, ppm)														
Nematode inoculum level (J₂/cm³ soil)	Plant height, cm				Shoot dry weight, g				Root dry weight, g				Gall index*	
	0	5	10	Mean N	0	5	10	Mean N	0	5	10	Mean N		
0	74.33	74.33	73.00	73.88	38.13	41.33	34.63	38.03	6.83	6.80	7.00	6.87	0.3	
2	67.00	62.66	55.16	61.61	35.96	33.73	29.33	33.01	6.26	5.13	4.63	5.34	2.4	
8	61.00	51.16	52.00	54.72	26.93	25.40	24.00	25.44	4.46	3.06	3.43	3.65	4.5	
Mean A	67.44	62.72	60.05		33.67	33.48	29.32		5.85	5.00	5.02		-	
Nematode (N) CD 0.05 P				3.48	3.51				0.60					-
Arsenic (A) CD 0.05 P				3.48	3.51				0.60					-
N X A CD 0.05 P				6.04	NS				NS					-

*Gall index using 0-5 scale



(A) HEIGHT (cm)

(B) WEIGHT (g)

Fig. 4.1.5. Effect of root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 1121. (A-Arsenic, N-Nematode)

Discussion

The content of major plant nutrients N, P and K was much reduced in the shoot of rice plants inoculated with *M. graminicola*. The reduction was more at higher inoculum level. This would indicate lesser uptake or translocation of these nutrients, which confirms the finding of Patil *et al.* (2013). The arsenic contamination of the soil at 5 and 10 ppm did not significantly affect the NPK content in shoot, although there were some indications of reduction, especially at the 10 ppm As concentration. The As contamination of soil up to the concentration tested also did not significantly reduce root galling due to *M. graminicola*. Although the nematode reproduction was not specifically studied this finding indicates no significant effect on *M. graminicola*. To the contrary, there was significant reduction in plant growth in terms of plant height and dry weights of root and shoot. It is interesting to note that the reduction in NPK content was negatively related to the inoculum level of *M. graminicola* but independent of the As contamination.

It was intriguing to see that whereas the NPK content in shoot was reduced, the content of As in root, shoot and grain was increased with inoculum level of *M. graminicola*. Even the reduction in root biomass did not prevent the translocation of As to the shoot. These findings prove that the root-knot nematode damage to root suppressed NPK uptake, but increased As uptake. This suggests that there would be different mechanisms of uptake and transport of NPK than that of Arsenic.

Miller and Cramer (2004) reported that reduced N uptake was primarily due to decreased fine roots growth caused by the presence of the sedentary nematode. Though the reduced uptake of nutrients was not directly linked to a fine root reduction, several histological reports showed that in roots infected by *Meloidogyne* there is an obstruction of the xylem as well as formation of non-functional vessels in the surrounding areas of the galls and giant cells (Dorhout *et al.*, 1991; Kirkpatrick *et al.*, 1991). Thus, nutrient transport could be affected by a decreased of water transport. Dorhout *et al.* (1988) used the dye Tinopal CBS to study water movement in the xylem of tomato roots infected with *M. incognita*. They observed that the dye leaked out of the xylem vessels close to the giant cells, remaining in the apoplast of the galls. They also observed that the water did not flow from the cortex to the stele along the nematode body, or from the stele to the cortex. Therefore, nutrients would also be retained in the roots. The reports on the influence of nematodes on N uptake by plants are controversial. Some reports detected an increase of N in the roots but no

alteration in the shoots (Hunter, 1958; Shafiee & Jenkins, 1963) while others showed that there was no alteration in the roots and shoots (Melakeberhan *et al.*, 1985; Goncalves *et al.*, 1995). However, none of these reports dealt with nutrients absorption along with heavy metals.

The preliminary study using clay loam soil from West Bengal indicated that As content in root was more than in shoot of 20 days rice seedlings. The nematode infection caused much increase in As content in both root and shoot. In the main experiments with loamy soil of New Delhi also, the concentration of heavy metal, As was significantly increased in shoots, roots and grains from healthy plants to higher inoculum levels and also had a positive correlation with increasing nematode inoculum levels. Though we have not studied the speciation of Arsenic, the previous reports have shown that the speciation of arsenic in the soil environment is dynamic; it can be biotically and abiotically inter-converted between the dominant solution phase inorganic species of less toxic arsenate and more toxic arsenite, the oxidized and reduced forms, respectively (Abedin *et al.*, 2002a). Both of them can be taken up by rice roots (Abedin *et al.*, 2002b). Iron plaque is commonly formed on the surfaces of roots of aquatic plants including rice by releasing oxygen to their rhizosphere through aerenchyma, resulting in the oxidation of ferrous iron to ferric iron, and the precipitation iron oxide on the root surface (Armstrong, 1964). The presence of iron plaque can sequester As and form a buffer zone that alters the entry of As into plants (Liu *et al.*, 2004). The presence of iron plaque can potentially alter the traditionally perceived phosphate-arsenate competition. Soil chemical transformation occurring under the flooded conditions is the main reason for the much enhanced As accumulation in paddy soil. Further studies are required to understand relationship of nematode, As uptake and enhanced As accumulation by the plant.

Mycorrhizal fungi and other microorganisms in the rhizosphere benefit the rice plants. There are many reports about effects of mycorrhizal fungi on As acquisition by plants. The fungi could restrict As translocation from root to shoot although the mechanism is not clear (Liu *et al.*, 2005; Chen *et al.*, 2007). Other bacteria, such as Ammonia-Oxidizing Archaea (AOA), Bacteria (AOB), iron reducing bacteria and sulphate reducing prokaryotes, are abundant in paddy soils (Chen *et al.*, 2008; Wang *et al.*, 2010; Liu *et al.*, 2009; Chen *et al.*, 2008). Some of them involved in iron reduction or oxidation in paddy soil probably cause transformation of As species,

leading to As co-precipitation with, or adsorption to, Fe (III) minerals in soil. Our results indicated that As uptake is increased from root to shoot because damaged root cortical cells by *M. graminicola* and galls acts as a nutrient sink, diverting nutrients to its giant cells may have predisposed the large number of accumulated As species in reduced state under anaerobic condition near the root zone. Thermodynamically, reduction of arsenate to arsenite can occur quite readily at intermediate redox potentials (Inskeep *et al.*, 2002). Flooding of paddy soils leads to mobilization of arsenite into the soil solution and enhanced As bioavailability to rice plants (Xu *et al.*, 2008). This is the reason why South East Asian countries like Bangladesh and West Bengal in India are severely affected by arsenic contamination in rice cultivation because of growing rice under submerged condition. Soil type is another factor, clay soil in these regions may retain the water more compared to Delhi region having alluvial soil that retains less water due to greater porosity.

Arsenic has a low mobility with respect to translocation from roots to shoots in rice. Arsenite appears to be the main As species that is transported from the root cortical cells to xylem vessels because in all of the plant species studied, arsenite is the predominant form of As in the xylem sap, accounting for 60-100% of total As. It is generally believed that As concentrations decrease in the order of roots > stems and leaves > husks > grain (Abedin *et al.*, 2002; Liu *et al.*, 2006). The high concentration of As in the husk is probably derived from the xylematic transport of As. Arsenite was translocated from root to shoot probably up to husk. By contrast, accumulation of As in the grain occur primarily through phloem. In our studies, the As uptake decreased from root > shoot > grains, which was in conformity with the reported trends that As species is transported from the root cortical cells to xylem vessels and disturbing of xylem vessels by *M. graminicola* may divert As translocation to grains via phloem cells (Zhao *et al.*, 2009).

These findings have shown that the risk of high As content in the shoot and grain of rice would be more if the plant roots are infected with root-knot nematode, *M. graminicola*. As may not directly interfere with uptake of NPK but may cause indirect effects through possible toxicity to the plant at high contamination levels. More studies are necessary to understand the differential uptake mechanism and effects of As on the plant and nematode metabolism and host-parasite relations. These findings also show that management of these nematodes is essential not only to

allow proper utilization of NPK and improving crop production but also to reduce the risk of increased content of toxic heavy metal like As in shoot, often used as fodder for livestock and grain, the staple food for humans.

Effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of macronutrients and Lead and plant growth of rice

Abstract

The rice root-knot nematode, *Meloidogyne graminicola* is considered one of the limiting factors in rice production in all rice ecosystems. Heavy metals are impurities in many chemical fertilizers, pesticides, insecticides and fungicides used in rice growing process. These can cause the accumulation of heavy metals in rice and when people consume rice, these toxic heavy metals may enter and deposit into fatty tissues. In this way, heavy metals may enter the food chain and affect human health. In the present study we carried out an experiment on the effect of the root-knot nematode, *M. graminicola*, a serious pest of rice, on the uptake of the major plant nutrients, viz., Nitrogen (N), Phosphorus (P) and Potassium (K) and the heavy metal, Lead (Pb) and growth of the *basmati* rice, *Oryza sativa*, cv. Pusa 1121. The results show that the content of N, P and K in rice shoot were significantly reduced and were negatively correlated to the nematode inoculum level. The plant height and shoot and root dry weights were also decreased with increasing nematode inoculum level. However, the heavy metal, Lead content in the plants 45 and 110 days after transplanting significantly (0.05 P) increased with increasing nematode inoculum level. Content of Pb in the plants grown in soil contaminated with 400 and 600 ppm Pb increased upto two times due to the nematode infection of roots, the increase being more at the higher inoculum level. In general, Pb contamination of soil upto 600 ppm did not cause significant reduction in plant growth or root galling due to *M. graminicola* but the data indicated possibility of toxicity to both plant and nematode at higher Pb contamination levels.

Key words: Nitrogen, Phosphorus, Potassium, Lead, Heavy metal, Soil pollution, *Meloidogyne graminicola*, Nematode, Rice.

Introduction

Plants are the target of a wide range of pollutants that vary in concentration, speciation, and toxicity. Such pollutants mainly enter the plant system through the soil (Arshad *et al.*, 2008) or via the atmosphere (Uzu *et al.*, 2010). Among common pollutants that affect plants, lead is one of the most toxic and frequently encountered (Cecchi *et al.*, 2008; Grover *et al.*, 2010; Shahid *et al.*, 2011). The sources include smelting, combustion of leaded gasoline, or applications of lead-contaminated media (sewage sludge and fertilizers) to land (Piotrowska *et al.*, 2009; Sammut *et al.*, 2010).

This metal impairs plant growth, root elongation, seed germination, seedling development, transpiration, chlorophyll production, lamellar organization in the chloroplast, and cell division (Sharma and Dubey, 2005; Krzesłowska *et al.*, 2009; Gupta *et al.*, 2009, 2010; Maestri *et al.*, 2010). Lead was reported as being the second most hazardous substance, after arsenic, based on the frequency of its occurrence, toxicity, and the potential for human exposure by the Agency for Toxic Substances and Disease Registry (ATSDR, 2003).

Rice is the most important staple food for about half of the human race. With the ever increasing pressure on food production and intensity of rice cultivation, proportionate compounding of pest and disease problems has been observed in several regions of the world. In fact, the preponderance of pest problems in rice is a major constraint in achieving a production revolution in rice comparable to wheat which has lesser pest problems in its growing season.

The root-knot nematode, *Meloidogyne graminicola* which are generally considered minor pests are gaining importance, as the economic losses caused by them are coming to light the world over. It has gained considerable attention during recent time because of extent of damage potential to rice. This nematode causes serious damage to irrigated and upland rice particularly under water stress conditions. An yield loss of about 21% in rainfed and well drained soil, and 16-32% in upland rice has been recorded due to the nematode infection (Dutta *et al.*, 2012). *M. graminicola* is found in these situations but causes serious damage when there is water stress. Both *M. graminicola* and *M. triticooryzae* can also multiply on wheat during warmer parts of the winter season and present higher initial population levels for the next rice crop (Gaur, 1993; Chandel *et al.*, 2002; Singh *et al.*, 2003). In the rice-wheat cropping system the nematode multiplies fast in rice and causes crop damage, while in the following wheat crop, on account of low winter temperature the

nematode damage is not apparent, but the population density of *M. graminicola* is sustained on wheat serving as a green bridge. Infested rice plants show less vigour, yellowing and curling of leaves. The chlorophyll content of leaves is reduced (Swain and Prasad, 1988).

Korcak and Fanning (1985) found a positive relationship between the concentration of Pb in the soil and that in the plant. In recent years there has been increasing awareness and concern over heavy metal contamination of soils and the effects this may be having on the food chain. The uptake of toxic heavy metals from contaminated soils by food and forage plants comprises a prominent path for such elements to enter the food chain and finally be ingested by humans. No reliable information is available on the effect of root-knot nematodes *M. graminicola* on uptake of Pb by the rice plants. The present studies were carried out to understand whether the uptake of Pb would follow same trend as reduced uptake of micro and macro nutrients due to increased nematode infestation levels or not. An investigation has been carried out on the effect of *M. graminicola* on the uptake of N, P, and K and heavy metal, Lead and plant growth of rice plants.

Materials and Methods

The effects of two inoculum levels of *M. graminicola* on the uptake of macronutrients, Nitrogen (N), Phosphorous (P) and Potassium (K) and heavy metal, Lead (Pb) and plant growth of rice have been studied under pot culture. Seeds of susceptible rice cv. Pusa 1121 were sown in steam sterilized soil in 25 cm diameter earthen pots. One month old seedlings were transplanted, four each in 25cm diameter plastic pots containing about 3000 cm³ autoclaved soil and sand mixture (3:1). One week after transplanting, the seedlings were inoculated with nematodes @ 0, 2, and 8 J₂ cm⁻³ of soil. All treatments were replicated four times and the pots were placed randomly in polygreenhouse at 27± 2°C. Uninoculated plants served as control. The pots were irrigated with deionised water and soil was kept near saturation.

In pot culture, the recommended dose of fertilizer, NPK were calculated and applied in soil before transplanting of rice. For heavy metal, Lead nitrate (Pb (NO₃)₂) was added to attain Pb (0 ppm, 400 ppm and 600 ppm), one month prior to transplanting of rice. For analysis of nutrients in the plant samples, total nitrogen content in plant was estimated in 1g dried samples by Kjeldhal method (Jackson, 1973). For phosphorus and potassium analysis, 1g dried plant samples were digested

with nitric and perchloric acids. Phosphorous was assessed by Spectrophotometer (UV 1800) using vanadate-molybdate reagent (Olsen *et al.* 1954). Available potassium was determined by using Flame Photometer (ME 881). Heavy metal, Lead (Pb) content in the plant samples were determined by Atomic Absorption Spectrophotometer (UV 1800).

The growth of plants was recorded on 110 days old plants in pot experiments. The plant height was measured with help of a scale taking ten randomly selected plants at the time of maturity. The plants after harvesting were cut into shoot and root portion separately for determining dry weights. Shoot and root portions were washed thoroughly with water for removing soil particles and other materials and kept in dry oven at 65°C for 72 h before recorded dry weight using electronic balance. Number of galls on roots per plant root system was also counted and used to assign root-knot indices.

Results

The Nitrogen content in the shoot of rice grown as control (nematode free soil without added Lead (Pb)) was 0.42%. Looking at the main effects, no significant changes due to application of 400-600 ppm Pb to soil but significant reduction in N content was observed in plants at *M. graminicola* inoculation levels of 8 J₂ cm⁻³ soil. The N content was reduced due to *M. graminicola* inoculation at the same levels with or without Pb treatment. Averaged over all the Pb levels, the N content was decreased from 0.42% to 0.38 (-17.4%) and 0.32 (-30.4%), respectively, at 2 and 8 J₂ cm⁻³ soil (Table. 4.2.1). However, the interaction effect of the nematode and Pb was not significant statistically at 0.05 P.

Similar trend was observed in the case of Phosphorus and Potassium contents of the shoot of rice, with minor variations. The main effect of the *M. graminicola* inoculation decreased the P content from 0.18 to 0.11 (-38.9 %) and 0.07 (-61.1) at the 2 and 8 J₂ cm⁻³ soil respectively. There was no reduction in the P content due to Pb at 0 and 2 J₂ cm⁻³ but at 8 J₂ cm⁻³, it was reduced from 0.09% in the absence of added Pb to 0.04% (-55.5%) at 600 ppm added Pb. Similarly, the main effect of *M. graminicola* in reducing the K content was significant. The average K content was 1.18 in the shoot of healthy plants; it was reduced significantly due to *M. graminicola* as well as due to added Pb to the soil. The effect of nematode being more severe. Averaged over the Pb levels, the K content was decreased from 1.01%

Table. 4.2.1. Effect of Lead and root-knot nematode, *Meloidogyne graminicola* on the macronutrients, NPK in shoot of rice cv. Pusa 1121

Nematode inoculum level (J ₂ /cm ³ soil)	Nitrogen (%)				Phosphorus (%)				Potassium (%)				
	Lead (ppm)			Mean N	Lead (ppm)			Mean N	Lead (ppm)			Mean N	
	0	400	600		0	400	600		0	400	600		
0	0.42	0.50	0.44	0.46	0.19	0.17	0.18	0.18	1.18	0.96	0.90	1.01	
2	0.39	0.40	0.36	0.38	0.13	0.11	0.09	0.11	0.76	0.69	0.70	0.71	
8	0.35	0.33	0.28	0.32	0.09	0.08	0.04	0.07	0.57	0.56	0.49	0.54	
Mean L	0.39	0.41	0.36		0.14	0.12	0.10		0.83	0.74	0.69		
Nematode (N) CD 0.05 P				0.03					0.02				
Lead (A) CD 0.05 P				NS					0.02				
N X L CD 0.05 P				NS					NS				

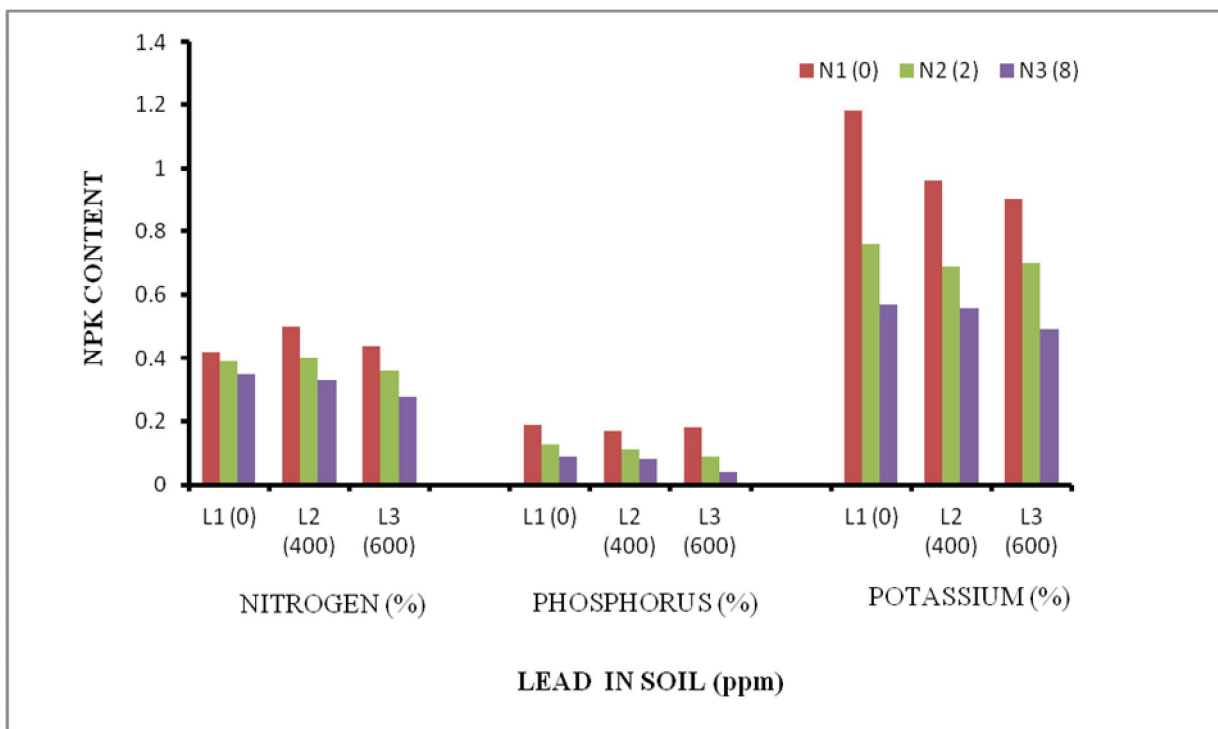


Fig. 4.2.1. Effect of Lead and root-knot nematode, *Meloidogyne graminicola* on the macronutrients, NPK in shoot of rice cv.Pusa 1121. (L- Lead, N- Nematode)

in healthy plants to 0.71% (-29.7%) and 0.54% (46.5%) at the 2 and 8 $J_2 \text{ cm}^{-3}$ soil, respectively (Table. 4.2.1, Fig. 4.2.1). The main effect of Pb on the K content was significant from the average 0.83% it was reduced to 0.74 (-10.8%) and 0.69 (16.9%) at the 400 and 600 ppm, respectively, the two levels being at par at 0.05. However, the interaction effects of Pb with nematode were non-significant statistically.

The focus of these investigations was mainly on Lead uptake; its content was studied in root and shoot at 45 and 110 days after transplanting (DAT). No Pb was detected in the root and shoot of rice cv. Pusa 1121 plants grown in sandy loam soil collected from IARI farm, New Delhi. The treatment of this soil @ 400 and 600 ppm resulted in 25.7 and 35.2 ppm Pb in root and 20.8 and 25.1 ppm in shoot, respectively, in the case of nematode free plants. In the case of *M. graminicola* 2 $J_2 \text{ cm}^{-3}$ soil inoculation, these levels increased to 38.9 and 50.1 ppm in root and 26.5 and 36.7 ppm in shoot, respectively. The increase in *M. graminicola* inoculation levels to 8 $J_2 \text{ cm}^{-3}$ soil resulted in further increase in Pb content, both in root and shoot (Table. 4.2.2, Fig. 4.2.2). The main as well as interaction effects of *M. graminicola* and Pb treatment levels were significant at 0.05 P, barring shoot uptake.

The main effect of *M. graminicola* inoculation at 2 and 8 $J_2 \text{ cm}^{-3}$ soil resulted in 46.1 and 79.2% increase in Pb content in root and 37.5 and 62.1% increase in shoot. In general, the Pb content was higher in root than in shoot. The increase in Pb content in both root and shoot were not in direct proportion of the increase in level of Pb contamination of soil or the inoculum level of *M. graminicola*. The interaction effect would show that proportionate increase in Pb contents of root and shoot were lesser at higher *M. graminicola* inoculum levels, although the Pb content was definitely higher than the lower nematode inoculum and Pb treatment level.

The observation at 110 DAT revealed that the Pb content in both root and shoot increased with age of plants, thus indicating Pb accumulation in plant tissues. More or less similar trends were observed at 110 DAT as at 45 DAT; with minor variations in proportion of increase in relation to Pb concentration and nematode inoculum levels. The increase in Pb content in grain was apparent. No Pb was detected in the grain harvested from plants grown in soil without Pb treatment. At 400ppm Pb contamination treatment of soil, 18.5ppm Pb was detected in the grain of nematode free plants; it increased to 20.3 and 38ppm at 2 and 8 $J_2 \text{ cm}^{-3}$ *M. graminicola* inoculation levels respectively. Similar trend was noticed at 600 ppm Pb contamination treatments. At 600 ppm Pb contamination treatment of soil and 8 J_2

Table. 4.2.2. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Lead uptake by rice cv. Pusa 1121

Lead uptake (ppm) (Initial observation, 45 Days After Transplanting)								
Nematode inoculum level (J₂/cm³ soil)	Root				Shoot			
	0	400	600	Mean N	0	400	600	Mean N
0	0.00	25.71	35.23	20.31	0.00	20.86	25.14	15.33
2	0.00	38.90	50.16	29.68	0.00	26.52	36.77	21.09
8	0.00	47.57	61.68	36.41	0.00	33.31	41.28	24.86
Mean L	0.00	37.39	49.02		0.00	26.90	34.40	
Nematode (N) CD 0.05 P				4.49	4.61			
Lead (L) CD 0.05 P				4.49	4.61			
N X L CD 0.05 P				7.79	NS			

Table. 4.2.3. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Lead uptake by rice cv. Pusa 1121

Lead uptake (ppm) (final observation, 110 DAT)													
Nematode inoculum level (J₂/cm³ soil)	Root				Shoot				Grain				
	0	400	600	Mean N	0	400	600	Mean N	0	400	600	Mean N	
0	0.00	16.89	22.44	13.11	0.00	15.93	25.21	13.71	0.00	18.57	23.54	14.03	
2	0.00	31.20	39.20	23.46	0.00	33.00	37.58	23.52	0.00	20.31	34.78	18.36	
8	0.00	49.11	63.54	37.55	0.00	38.05	49.94	29.33	0.00	37.98	41.80	26.59	
Mean L	0.00	32.40	41.72		0.00	28.99	37.58		0.00	25.62	33.37		
Nematode (N) CD 0.05 P				5.11					4.57				
Lead (L) CD 0.05 P				5.11					4.57				
N X L CD 0.05 P				8.85					7.92				

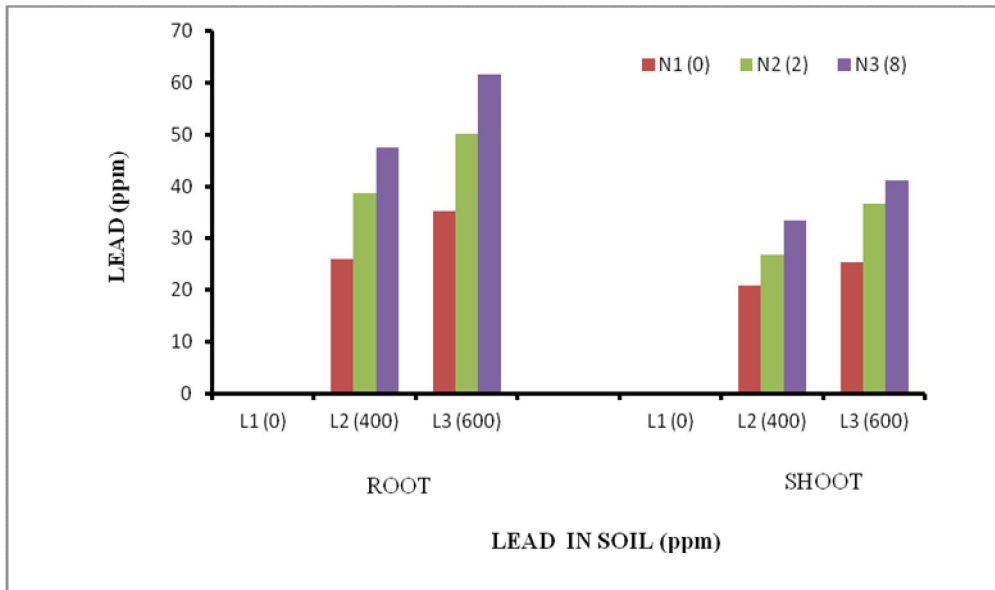


Fig. 4.2.2. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Lead uptake by rice cv. Pusa 1121, 45 days after transplanting. (L-Lead, N-Nematode)

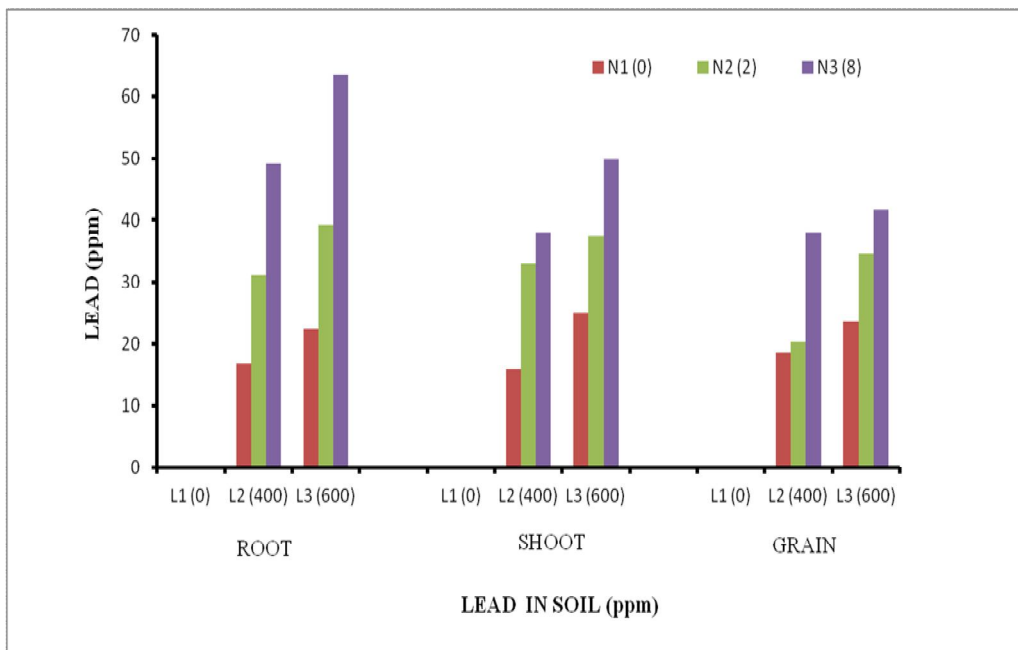


Fig. 4.2.3. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Lead uptake by rice cv. Pusa 1121, 110 days after transplanting. (L- Lead, N- Nematode)

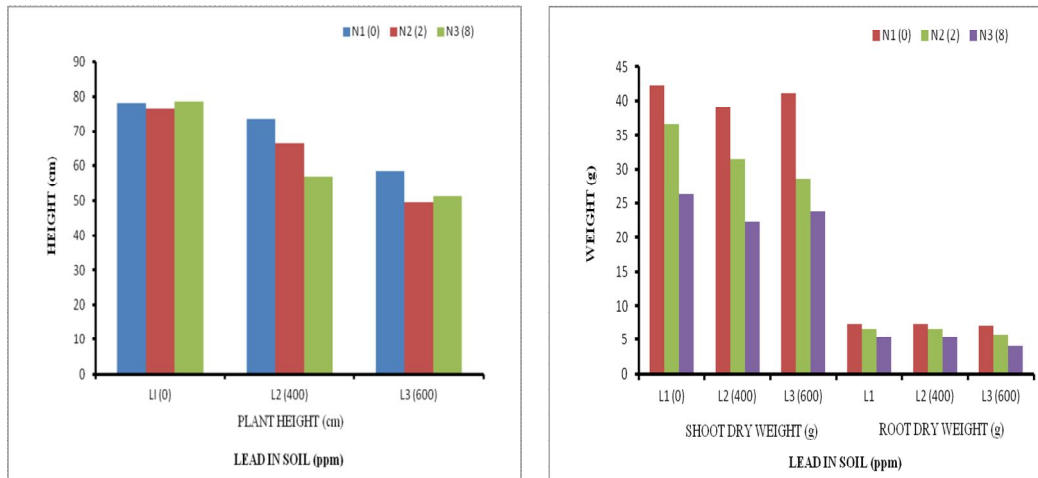
cm⁻³ *M. graminicola* inoculum, the Pb content in the grain as high as 41.8 ppm (Table. 4.2.3, Fig. 4.2.3).

The effect of *M. graminicola* inoculum was apparent in the reduced shoot height and dry weights of root and shoot. The root dry weight was decreased by 19.1 and 38.3%; shoot height by 15.3 and 19.2% and shoot dry weights by 21 and 41%, respectively at *M. graminicola* inoculum levels of 2 and 8 J₂ cm⁻³ soil. The main effect of Pb on the plant growth was significant (0.05P) reduction in root dry weight (12.4 and 15.5%), plant height (8.4 and 11.1%) barring shoot dry weight (11.8 and 11.1%) which is non-significant (Table. 4.2.4, Fig. 4.2.4). The interaction effect of Pb and *M. graminicola* on plant growth characters was not significant at 0.05P. The number of galls on the root system was significantly higher though not in the same ratio at the inoculum level of 8 J₂ cm⁻³ compared to the lower levels of 2 J₂ cm⁻³ soil. However, the Pb contamination of the soil at 400 or 600 ppm did not cause reduction in root galling.

Table. 4.2.4. Effect of Lead and root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 1121

Plant growth parameters, 110 DAT (Lead treated soil, ppm)															
Nematode inoculum level (J₂/cm³ soil)	Plant height, cm				Shoot dry weight, g				Root dry weight, g				Gall index*		
	0	400	600	Mean N	0	400	600	Mean N	0	400	600	Mean N			
0	78.16	76.33	78.66	77.72	42.13	39.03	41.06	40.74	7.30	7.10	7.20	7.20	0.5		
2	73.66	66.66	57.00	65.77	36.63	31.40	28.63	32.22	6.63	5.73	5.10	5.82	2.8		
8	58.50	49.50	51.33	53.11	26.30	22.23	23.73	24.08	5.33	4.03	3.96	4.44	4.5		
Mean L	70.11	64.16	62.33		35.02	30.88	31.14		6.42	5.62	5.42		-		
Nematode (N) CD 0.05 P				5.72					4.18					0.74	-
Lead (L) CD 0.05 P				5.72					NS					0.74	-
N X L CD 0.05 P				NS					NS					NS	-

*Gall index using 0-5 scale



(A) HEIGHT (cm)

(B) WEIGHT (g)

Fig. 4.2.4. Effect of Lead and root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 112. (L-Lead, N-Nematode)

Discussion

Our investigation shows that the content of N, P and K in rice shoots was significantly reduced and was negatively correlated to the nematode inoculum level. The plant height, shoot and root dry weight were also decreased with increasing nematode inoculum level. These results indicate that concentration of nutrients was reduced in plants and plant growth was suppressed by nematode infection. However, the Lead content in the plants was increased with increasing nematode inoculum level.

The contradictory results in the literature have been shown for root-knot nematode effects on by plants. Some authors reported that an increase of N in the roots but no alteration in the shoots (Hunter, 1958; Shafiee & Jenkins, 1963) while others showed that there was no alteration in the roots and shoots (Melakeberhan *et al.*, 1985; Goncalves *et al.*, 1995). However, none of these reports dealt with nutrients absorption along with heavy metals were done. Several histological reports showed that in roots infected by *Meloidogyne* there is an obstruction of the xylem as well as formation of non functional vessels in the surrounding areas of the galls and giant cells (Dorhout *et al.*, 1991; Kirkpatrick *et al.*, 1991). Thus, nutrient transport could be affected by a decrease of water transport. Our result clearly indicated that, mobilization of nutrients from soil to plant diverted by *M. graminicola* to its giant cells which acts as a nutrient sink. Diverted nutrients are utilized for their growth, subsequently the plants shows wilting symptoms because of blocking of water transport in xylum vessels by galls. Infected plants had reduced uptake of nutrients from soil to shoot part.

The process of Pb taken up from soils by roots is strongly governed by plant factors, and different plant species own different properties in Pb uptake and distribution. Some are root-accumulator, storing Pb in the roots, transporting little to above ground parts, and others are shoot-accumulators, storing greatly more Pb in the shoots (Welsh and Denny, 1980). Plant factors, such as root surface area, root exudates, mycorrhization, and the rate of transpiration affect the availability of Pb in soil and the uptake of Pb by plants (Davies *et al.*, 1995). The differences among the rice cultivars in the uptake of Pb from soil may result from the differences in their root absorption abilities and exudates properties. The differences in the translocation of Pb in rice plants are possibly related to the differences in the forms of Pb existed in the plants. It was reported that the ions and low-molecule complexes of Pb are mobile

in plants (Yang *et al.*, 1993). Liu *et al.* 2003 reported that the Pb concentrations decreased sharply from roots to grains along rice plants, so the concentrations of Pb were very low in grains compared to other parts of rice plants, and there were significant and negative correlations between connected organs of rice plant for the Pb concentrations in them. Our findings conformed to the view that Pb accumulation was higher in the roots and decreased from roots to grains but the accumulation rate may vary depending on the rice cultivars. Pb moves predominantly into the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of Pb between root and shoot. This may in part account for the reports of higher accumulation of Pb in roots compared to shoots (Verma and Dubey, 2003). Plant factor is majorly responsible for translocation of Pb in one hand and root-knot nematodes damage to the cortical cells and xylem vessels also responsible for predisposing the entry of Pb to the roots on the other hand.

It can be concluded that the risk of high Pb content in the shoot and grain of rice would be more if the plant roots are infected with root-knot nematode, *M. graminicola*. The Pb may not directly interfere with uptake of NPK but may cause indirect effects through possible toxicity to the plant at high contamination levels. Special attention should be given in future to the question of the metal complexes formed inside plants and the metabolism of those metal-complexes in the food chain, differential uptake mechanism and effects of Pb on the plant and nematode metabolism and host-parasite relations.

Effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of macronutrients and Cadmium and plant growth of rice

Abstract

Rice root-knot nematode, *Meloidogyne graminicola* has attained wide importance due to its potential to cause major damage in rice-wheat cropping system. Heavy metals are common environmental contaminants arising from or associated with metal mining and numerous other industrial, urban and agricultural activities. Investigations have been carried out on the effect of *M. graminicola*, on the content of the major plant nutrients, viz. Nitrogen (N), Phosphorus (P) and Potassium (K) and the heavy metal, Cadmium (Cd) and growth of the *basmati* rice, *Oryza sativa*, cv. Pusa 1121. The content of NPK after 45 and 110 days after transplanting was significantly lower in the shoot of rice inoculated with 2 and 8 J₂ cm⁻³ soil compared to nematode-free plants, the reduction being more at the higher inoculum level. The N content was decreased from -18.7% and -35.4%, respectively, at 2 and 8 J₂ cm⁻³ soil. Similar trend was observed in the case of Phosphorus and Potassium contents of the shoot of rice, with minor variations. To the contrary, the content of Cd in the plants grown in soil contaminated with 3 and 6 ppm Cd was increased upto three times due to the nematode infection of roots, the increase being more at the higher inoculum level. The Cd content in plant was more at the higher soil contamination level. The plant height, shoot and root dry weight also decreased with increasing nematode inoculum level. The root dry weight was decreased by 32.7 and 51%; shoot height by 8.8 and 30.6% and shoot dry weight by 16.8 and 24.6%, respectively, at *M. graminicola* inoculum levels of 2 and 8 J₂ cm⁻³ soil. The results confirmed that *M. graminicola* infection of root may not only reduce uptake of NPK and decrease plant growth but can also increase the uptake of the toxic heavy metal, Cadmium, a definite health hazard for humans and other consumers.

Key words: Nitrogen, Phosphorus, Potassium, Cadmium, Heavy metal, Soil pollution, *Meloidogyne graminicola*, Nematode, Rice.

Introduction

The root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield, 1965 is known to infect and cause serious damage to cereals, especially rice, in many countries (Arayarungsarit, 1987; Bridge, 1990; Plowright and Bridge, 1990; Prot and Matias, 1995; Padgham *et al.*, 2004; Pokharel *et al.*, 2007, Dutta *et al.*, 2012). It is a serious problem in the nurseries and upland rice but has been recently found to be widespread in the deepwater and irrigated rice also, in many states of India (Prasad *et al.*, 1985, 1986; Rao *et al.*, 1986; MacGowan, 1989; Bridge *et al.*, 1990; Jairajpuri and Baqri, 1991, Gaur & Pankaj, 2010). The root-knot nematodes, *M. graminicola* and *M. triticooryzae*, infecting rice and wheat also cause serious losses to rice crops in some areas in north India (Gaur *et al.*, 1993, 1996). *M. graminicola* is the most common RKN species infecting rice. In India, it is reported to cause 17-30% yield loss due to poorly filled kernels (MacGowan, 1989; Jain *et al.*, 2007).

Rice is not only a staple food but also commercially important; especially in the basmati rice which is a premium product having high market demand in India as well as for export. Therefore, quantities of produce as well as quality are important factors determining production and nutritional and commercial value. Infected root tips become swollen and hooked, a symptom characteristic of this nematode species. In upland conditions and shallow intermittently flooded land, *M. graminicola* is considered to be by far the most damaging *Meloidogyne* species on rice. Histological studies have shown disruption of the xylum tissues in *Meloidogyne* galls as well as the presence of abnormally formed xylum vessels causing interruption of induced root water transport (Kirkpatrick *et al.*, 1991) and consequently the translocation of absorbed nutrients to the shoot. Patil *et al.* (2013) have shown reduction in uptake of macro nutrients, Nitrogen (N), Phosphorus (P), Potassium (K) and micro nutrients, Iron (Fe) and Zinc (Zn) due to *M. graminicola* infection of the roots.

Cadmium is ubiquitous and potentially hazardous contaminant in the biosphere (Zaman and Zereen, 1998). Phosphatic fertilizers are widely regarded as being the most common source of Cd contamination of agricultural soils (Alloway, 1995). Cadmium is readily taken up by plant roots and translocated to above ground tissues (Yang *et al.*, 1998) and then poses a potential threat to human health as it enters the food chain (Obata and Omebayashi, 1997). No reliable information is available on the effect of *M. graminicola* on uptake of Cd by the rice plants. The

present studies were carried out to understand whether the uptake of Cd would follow same trend as reduced uptake of micro and macro nutrients due to increased nematode infestation levels or not.

Materials and Methods

The effects of a series of inoculum levels of *M. graminicola* on the uptake of macronutrients, Nitrogen (N), Phosphorous (P) and Potassium (K) and heavy metal, Cadmium (Cd) and plant growth of rice have been studied under pot culture. Seeds of susceptible rice cv. Pusa 1121 were sown in steam sterilized soil in 25 cm diameter earthen pots. One month old seedlings were transplanted, four each in 25 cm diameter plastic pots containing about 3000 cm³ autoclaved soil and sand mixture (3:1). One week after transplanting, the seedlings were inoculated with nematodes @ 0, 2 or 8 J₂ cm⁻³ of soil. All treatments were replicated four times and the pots were placed randomly in polygreenhouse at 27± 2°C. Uninoculated plants served as control. The pots were irrigated with deionised water and soil was kept near saturation.

In pot culture, the recommended dose of fertilizer, NPK were calculated and applied in soil before transplanting of rice to be done. For heavy metal, Cadmium nitrate (Cd (NO₃)₂.4 H₂O) was added to attain Cd (0ppm, 3ppm and 6ppm) one month prior to transplanting of rice. For analysis of nutrients in the plant samples, total nitrogen content in plant was estimated in 1g dried samples by Kjeldhal method (Jackson, 1973). For phosphorus and potassium analysis, 1g dried plant samples were digested with nitric and perchloric acids. Phosphorus was assessed by Spectrophotometer (UV 1800) using vanadate-molybdate reagent (Olsen *et al.*, 1954). Available potassium was determined by using Flame Photometer (ME 881). Heavy metal, Cadmium (Cd) content in the plant samples was determined by Atomic Absorption Spectrophotometer (UV 1800).

The growth of plants was recorded on the 110 days old plants in pot experiments. The plant height was measured with help of a scale taking ten randomly selected plants at the time of maturity. The plants after harvesting were cut into shoot and root portions separately for determining dry weights. Shoot and root portions were washed thoroughly with water for removing soil particles and other materials and kept in dry oven at 65°C for 72 h were recorded using electronic weighing machine. No of galls on roots per plant root system was also counted.

Result

In the healthy plant (nematode and heavy metal, Cadmium free soil), the N content in the shoot of rice was 0.45%. No significant changes due to 3-6ppm Cd to soil but slight reduction in N content was observed in plants at *M. graminicola* inoculum level of 8 J₂ cm⁻³ soil. The differences were not statistically significant at 0.05P. The main effect of Cd on the N content was not significant (Table. 4.3.1, Fig. 4.3.1). On the other side, the N content was drastically reduced due to *M. graminicola* inoculum level of 2 and 8 J₂ cm⁻³ soil with or without Cd treatment. Averaged over all the Cd levels, the N content was decreased from 0.48% to 0.39 (-18.7%) and 0.31(-35.4%) respectively, at 2 and 8 J₂ cm⁻³ soil. Thus, the main effect of *M. graminicola* inoculation in reducing N content of shoot was significant (0.05P). However, the interaction effect of the nematode and Cd was non-significant statistically.

The same trend was continuing in the case of Phosphorus and Potassium contents of the shoot of rice, with minor variation (Table 4.3.1, Fig. 4.3.1). The P content was decreased from 0.18 to 0.12 (-33.3%) and 0.08 (-55.5%) at *M. graminicola* inoculum level of 2 and 8 J₂ cm⁻³ soil. Thus, the main effect of *M. graminicola* inoculation in reducing N content of shoot was significant (0.05P). There were no reduction in the P content at 0 and 2 J₂ cm⁻³ soil but at 8 J₂ cm⁻³ soil, it was 0.10% in the absence of added Cd and reduced to 0.04% (-60%). The main effect of Cd and its interaction with nematode were significant statistically (0.05P). Similarly, the main effect of *M. graminicola* in reducing the K content was significant. The average K content was 1.01% in the shoot of healthy plants; it was reduced greatly due to *M. graminicola* but only slightly due to the added Cd to the soil. Averaged over the Cd levels, the K content was decreased from 0.92% in healthy plants to 0.76 (-17.4%) and 0.56% (-39.1%) at the 2 and 8 J₂ cm⁻³ soil, respectively. The main effect of Cd as well as the interaction with nematode were non-significant statistically.

Since the focus of these investigations was more on Cadmium uptake, its content was studied in root and shoot at 45 and 110 days after transplanting. No Cd was detected in the root or shoot of rice cv. Pusa 1121 plants grown in sandy loam soil collected from IARI farm, New Delhi. The treatment of this soil @ 3 and 6ppm resulted in 0.44 and 1.14ppm Cd in root and 0.27 and 0.87ppm in shoot, respectively,

Table. 4.3.1. Effect of Cadmium and root-knot nematode, *Meloidogyne graminicola* on the macro nutrients, NPK in shoot of rice cv.Pusa 1121.

Nematode inoculum level (J ₂ /cm ³ soil)	Nitrogen (%)				Phosphorus (%)				Potassium (%)				
	Cadmium (ppm)			Mean N	Cadmium (ppm)			Mean N	Cadmium (ppm)			Mean N	
	0	3	6		0	3	6		0	3	6		
0	0.45	0.52	0.46	0.48	0.19	0.16	0.20	0.18	1.01	0.89	0.85	0.92	
2	0.41	0.39	0.39	0.39	0.17	0.12	0.09	0.12	0.77	0.77	0.74	0.76	
8	0.33	0.34	0.27	0.31	0.10	0.09	0.04	0.08	0.56	0.55	0.59	0.56	
Mean C	0.40	0.41	0.37		0.15	0.12	0.11		0.78	0.73	0.73		
Nematode (N) CD 0.05 P				0.03					0.01				
Cadmium (C) CD 0.05 P				NS					0.01				
N X C CD 0.05 P				NS					0.03				

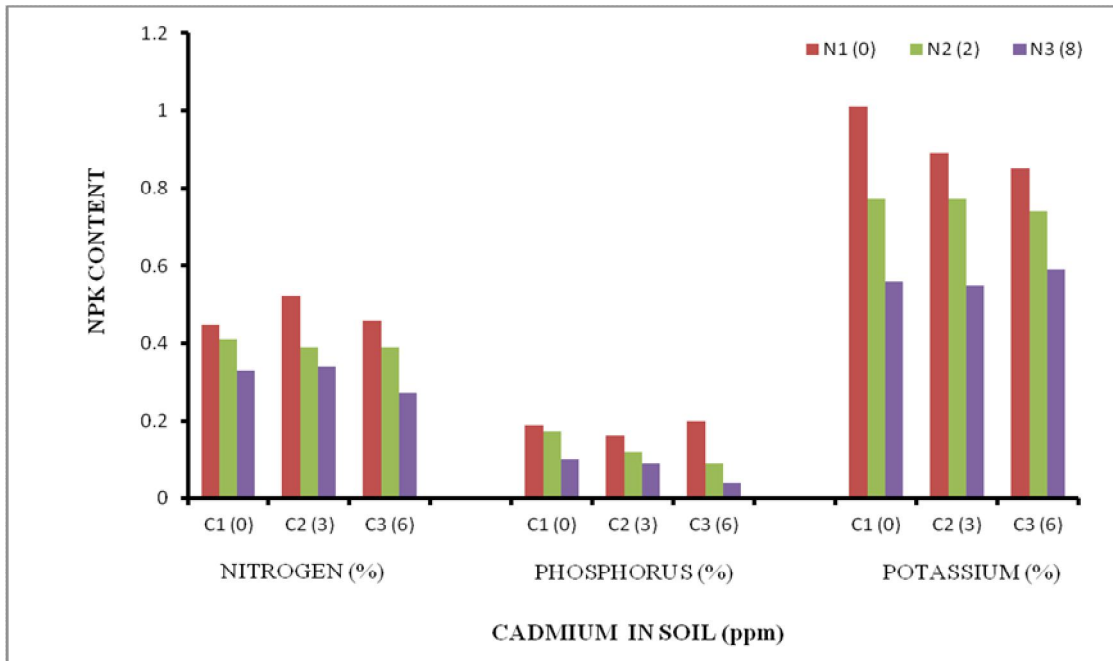


Fig. 4.3.1. Effect of Cadmium and root-knot nematode, *Meloidogyne graminicola* on the macro nutrients, NPK in shoot of rice cv. Pusa 1121. (C(x) – Cadmium (ppm), N(x) - Nematode(J2 cm⁻³))

in the case of nematode free plants. In the case of *M. graminicola* 2 J₂ cm⁻³ soil inoculation, these levels increased to 1.11 and 1.46 ppm in root and 0.52 and 1.13ppm in shoot, respectively. The increase in *M. graminicola* inoculum levels to 8 J₂ cm⁻³ soil resulted in further increased in Cd content, both in root and shoot (Table 4.3.2, Fig. 4.3.2). The main as well as interaction effects of *M. graminicola* and Cd treatments were significant at 0.05P in root content barring shoot content.

The main effect of *M. graminicola* inoculation at 2 and 8 J₂ cm⁻³ soil resulted in 65.3 and 215.3% increase in Cd content in root, 44.7 and 129% increase in shoot. In general the Cd content was higher in root than in shoot. The increases in Cd content in both root and shoot were not in direct proportion of the increase in levels of Cd contamination of soil or the inoculum level of *M. graminicola*. increase in added Cd from 3 to 6ppm resulted in 54.6 and 83% increase in Cd content of root and shoot, respectively, averaged over the *M. graminicola* inoculum levels. Similarly, the increase in inoculum level from 2 to 8 J₂ cm⁻³ soil resulted in 90.6 and 58.1% increase in Cd content of root and shoot, respectively. The interaction effect would show that proportionate increase in Cd contents of root and shoot were lesser at higher *M. graminicola* inoculum levels, although the Cd content was definitely higher than the lower nematode inoculum and Cd treatment level.

The observation at 110 DAT revealed that the Cd content in both root and shoot increased with age of plants, thus indicating Cd accumulation in plant tissues. More or less similar trend were observed at 110 DAT as at 45 DAT; with minor variations in proportionate increase in relation to Cd concentration and nematode inoculum levels. The increase in Cd content in grain was apparent. No Cd was detected in the grain harvested from plants grown in soil without Cd treatment. At 3 ppm Cd contamination treatment of soil, 0.30 ppm Cd was detected in the grain of nematode free plants; it increased to 0.62 and 0.90ppm at 2 and 8 J₂ cm⁻³ *M. graminicola* inoculum levels. To the contrary, the trend was reversed at 6ppm Cd contamination treatment, it decreased from 1.23 to 1.19ppm at 2 and 8 J₂ cm⁻³ *M. graminicola* inoculum levels (Table 4.3.3, Fig. 4.3.3).

The effect of *M. graminicola* inoculum was apparent in the reduced plant growth of rice measured in terms of shoot height and dry weights of root and shoot. The root dry weight was decreased by 32.7 and 51%; shoot height by 8.8 and 30.61% and shoot dry weight by 16.8 and 24.6%, respectively at *M. graminicola* inoculum levels of 2 and 8 J₂ cm⁻³ soil (Table 4.3.4, Fig. 4.3.4). The number of galls on the root

Table. 4.3.2. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Cadmium uptake by rice cv. Pusa 1121

Cadmium uptake (ppm) (Initial observation, 45 Days After Transplanting)								
Nematode inoculum level (J₂/cm³ soil)	Root				Shoot			
	0	3	6	Mean N	0	3	6	Mean N
0	0.00	0.44	1.14	0.52	0.00	0.27	0.87	0.38
2	0.00	1.11	1.46	0.86	0.00	0.52	1.13	0.55
8	0.00	2.02	2.91	1.64	0.00	1.12	1.50	0.87
Mean C	0.00	1.19	1.84		0.00	0.64	1.17	
Nematode (N) CD 0.05 P				0.42	0.31			
Cadmium (A) CD 0.05 P				0.42	0.31			
N X C CD 0.05 P				0.74	NS			

Table. 4.3.3. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Cadmium uptake by rice cv. Pusa 1121

Cadmium uptake (ppm) (final observation, 110 DAT)													
Nematode inoculum level (J₂/cm³ soil)	Root				Shoot				Grain				
	0	3	6	Mean N	0	3	6	Mean N	0	3	6	Mean N	
0	0.00	0.60	0.98	0.52	0.00	0.41	0.90	0.43	0.00	0.30	0.49	0.26	
2	0.00	1.02	1.35	0.79	0.00	0.72	1.23	0.65	0.00	0.62	1.23	0.61	
8	0.00	1.76	2.06	1.27	0.00	1.83	2.03	1.28	0.00	0.90	1.19	0.69	
Mean C	0.00	1.13	1.46		0.00	0.98	1.39		0.00	0.60	0.97		
Nematode (N) CD 0.05 P				0.47					0.59	NS			
Cadmium (A) CD 0.05 P				0.47					0.59	0.45			
N X C CD 0.05 P				NS					NS	NS			

Table. 4.3.4. Effect of Cadmium and root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 1121

Plant growth (110 DAT) Cadmium treated soil (ppm)															
Nematode inoculum level (J₂/cm³ soil)	Plant height, cm				Shoot dry weight, g				Root dry weight, g				Gall index*		
	0	3	6	Mean N	0	3	6	Mean N	0	3	6	Mean N			
0	72.66	74.50	77.66	74.94	35.30	32.16	39.00	35.48	7.73	7.60	7.30	7.54	0.5		
2	74.33	69.00	61.66	68.33	33.96	29.36	25.16	29.50	6.03	5.36	3.83	5.07	3.2		
8	60.00	48.33	47.66	52.00	31.20	26.33	22.63	26.72	4.86	3.33	2.90	3.70	4.3		
Mean C	69.00	63.94	62.33		33.48	29.28	28.93		6.21	5.43	4.67		-		
Nematode (N) CD 0.05 P				3.98					3.56					0.67	-
Cadmium (C) CD 0.05 P				3.98					3.56					0.67	-
N X C CD 0.05 P				6.90					6.18					NS	-

*Gall index using 0-5 scale

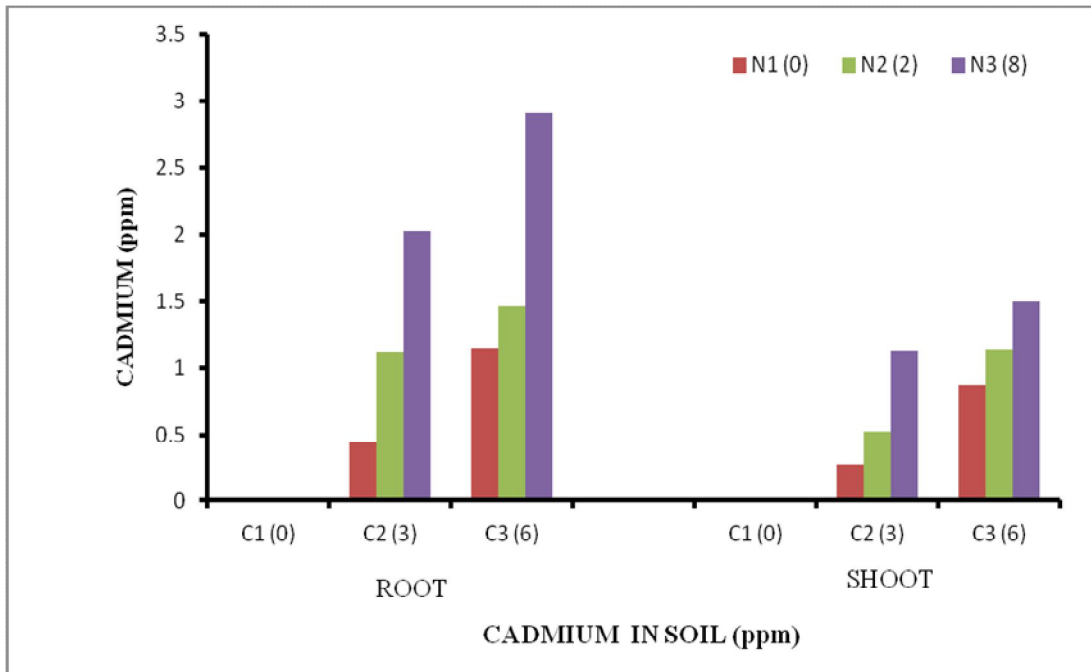


Fig. 4.3.2. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Cadmium uptake by rice cv. Pusa 1121, 45 days after transplanting. (C- Cadmium, N-Nematode)

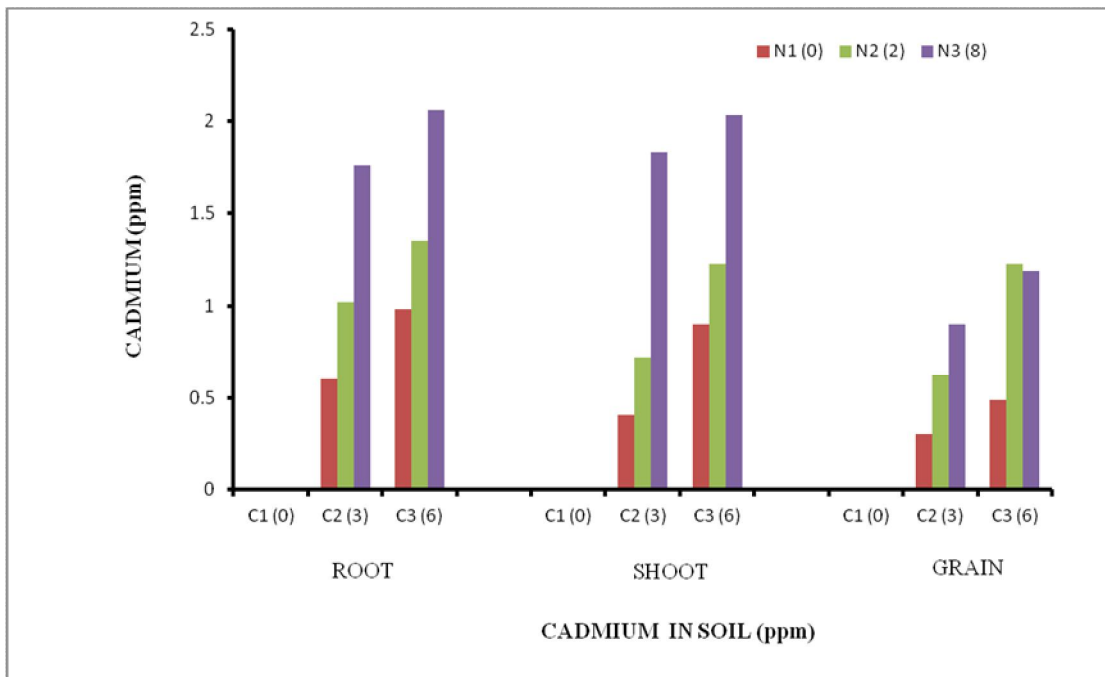
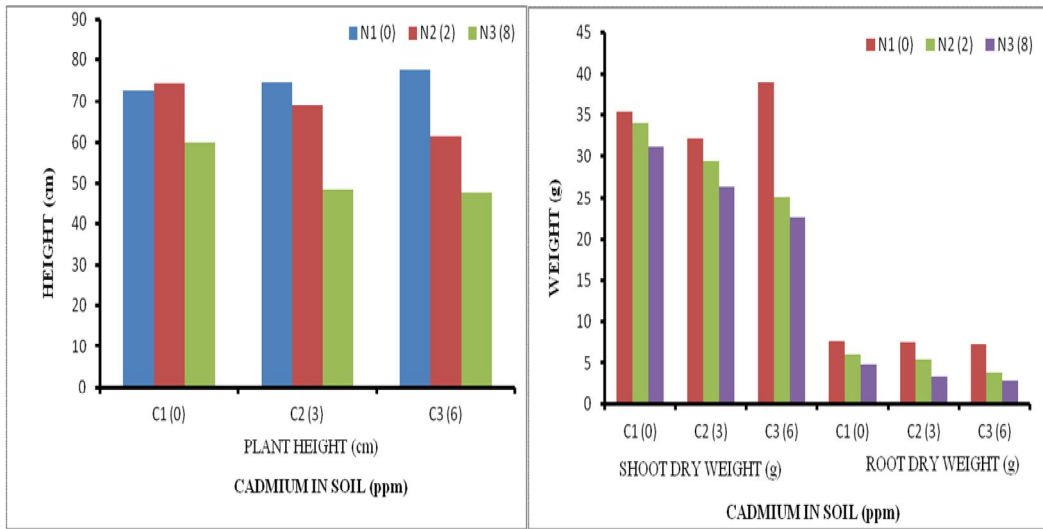


Fig. 4.3.3. Effect of root-knot nematode, *Meloidogyne graminicola* on heavy metal, Cadmium uptake by rice cv. Pusa 1121, 110 days after transplanting. (C- Cadmium, N-Nematode)



(A)

(B) WEIGHT (g)

HEIGHT (cm)

Fig. 4.3.4. Effect of root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 1121. (C- Cadmium, N-Nematode)

system was significantly higher though not in the same ratio at the inoculum level of $8 \text{ J}_2 \text{ cm}^{-3}$ compared to the lower levels of $2 \text{ J}_2 \text{ cm}^{-3}$ soil. However, the Cd contamination of the soil at 5 or 10 ppm did not cause reduction in root galling.

Discussion

The present study shows that the concentrations of N, P and K in rice leaves were significantly reduced and were negatively correlated to the nematode inoculum level. The plant height, shoot and root dry weight were also decreased with increasing nematode inoculum level. These results indicate that concentration of nutrients was reduced in plants and their growth was suppressed by nematode infection. This would indicate lesser uptake or translocation of these nutrients, which confirms the findings of Patil *et al.* (2013). However, the amount of Cd accumulated was generally greater in the plants inoculated with high level of *M. graminicola* than in the uninoculated plants. Though the reduced uptake of nutrients was not directly linked to a fine root reduction, several histological reports showed that in roots infected by *Meloidogyne* there is an obstruction of the xylem as well as formation of non-functional vessels in the surrounding areas of the galls and giant cells (Dorhout *et al.*, 1991; Kirkpatrick *et al.*, 1991). The data on the influence of nematodes on N uptake by plants are controversial. Some reports detected an increase of N in the roots but no alteration in the shoots (Hunter, 1958; Shafiee & Jenkins, 1963) while others showed that there was no alteration in the root and shoot (Melakeberhan *et al.*, 1985; Goncalves *et al.*, 1995). However, none of these reports dealt with nutrients absorption along with heavy metals.

Cadmium accumulated in plants can interfere with several physiological processes resulting in low productivity (Obata and Omebayashi, 1997). The excess amount of Cd in soil caused disturbances in mineral nutrition and carbohydrate metabolism (Moya *et al.*, 1993). This is in conformity with our reports, that the reduction of macronutrients was more at higher nematode inoculum levels at $8 \text{ J}_2 \text{ cm}^{-3}$ soil with higher amount of Cd. Jiang *et al.* (2004) also reported that Cd accumulation in both shoots and roots of Indian mustard increased with increasing soil Cd treatments. It may be concluded that metal accumulation in plants infected with root knot nematodes may depend upon the metal concentration in the soil and the population density of root-knot nematode. Further studies are required to understand relationship of nematode, Cd uptake and enhanced Cd accumulation by

the plant, including the possible differences in mechanisms of uptake and accumulation of the macronutrients and heavy metals.

The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis (Padmaja *et al.*, 1990). In our case the plant height, shoot and root dry weight were also decreased with increasing nematode inoculum level as well as higher amount of Cd. The ability of roots to absorb Cd may depend on both the activity of roots and the interaction between roots and its located soil environment. The proportions of soluble or bio-available Cd and minerals vary considerably with the redox potential in paddy soil, corresponding to the reduction of sulfate to sulfides, binding to organisms, binding with iron and manganese oxides, and absorption by soil granules (Kashem and Singh, 2001). Different rice cultivars possess different redox abilities in root, thus creating different rhizospheric environments and accessing different amounts of plant-available Cd and mineral nutrients, especially in submerged paddy soil (Liu *et al.*, 2000).

Kibria *et al.* 2006 reported that Cd concentration in shoot and root increased with increasing Cd treatments in all the three soils, viz. clay loam, sandy clay loam and sandy loam soil in rice. Eriksson (1989) who found that for the same total Cd content, Cd was more soluble and more plant available in sandy soil than clay soil. However, in the present study, Cd content in rice grain was higher in sandy clay loam because South East Asian countries like Bangladesh and West Bengal in India are severely affected by arsenic, lead and cadmium contamination in rice cultivation because of growing rice under submerged condition. Soil type is another factor, clay soil in these regions may retain the water more compared to Delhi region having alluvial soil that retains less water due to greater porosity.

This study may help in understand the effects of heavy metal pollution in the soil eco-system and its effects on plant growth, which are suitable indicators for assessing soil health. These findings have shown that the risk of high Cd content in the shoot and grain of rice would be more if the plant roots are infected with root-knot nematode, *M. graminicola*. The Cd may not directly interfere with uptake of NPK but may cause indirect effects through possible toxicity to the plant at high contamination levels. More studies are necessary to understand the differential uptake mechanism and effects of Cd on the plant and nematode metabolism and host-parasite relations.

Effect of varying population density of root-knot nematode, *Meloidogyne graminicola* on the uptake of macronutrients and arsenic and lead in rice, *Oryza sativa* under field condition

Abstract

Rice root-knot nematode, *Meloidogyne graminicola* has attained wide importance due to its potential to cause major damage in rice-wheat cropping system. Rice (*Oryza sativa*) accumulates the highest amount of As of all grain crops, largely because of the high plant availability of As under reduced soil conditions. Similarly, Lead (Pb) also causes a serious problem as the levels of Pb in soil that are toxic to plant are not easy to evaluate. Investigations have been carried out on the effect of the root-knot nematode, *Meloidogyne graminicola*, a serious pest of rice, on the content of the major plant nutrients, viz. Nitrogen (N), Phosphorus (P) and Potassium (K) and the heavy metal, Arsenic (As) and Lead (Pb) and growth of the *basmati* rice, *Oryza sativa*, cv. Pusa 1121. The content of NPK after 110 days after transplanting was significantly lower in the shoot of rice at high nematode infestation level. The concentration of all the three elements was negatively correlated to nematode infestation levels in the field. The heavy metals, Arsenic (As) and Lead (Pb) was detected in the root, shoot and grains of rice plants. The increase in *M. graminicola* infestation to high level resulted in further increased in As and Pb content, both in root and shoot observed in both plot 1&2. The concentration of As and Pb in grains was also found significantly higher in nematode infected plants. The heavy metal uptake by plants was positively correlated to the nematode infestation level in plots 1&2. The plant height, shoot dry weight and root dry weight were seen strong negatively correlated to the nematode infestation level. The results confirmed that *M. graminicola* infection of root may not only reduce uptake of NPK and decrease plant growth but can also increase the uptake of the toxic heavy metals, Arsenic and Lead, a definite health hazard for humans and other consumers.

Key words: Nitrogen, Phosphorus, Potassium, Arsenic, Lead, Heavy metal, Soil pollution, *Meloidogyne graminicola*, Nematode, Rice.

Introduction

Heavy metal pollution of the environment has become a growing ecological crisis and concern and therefore, the subject of much research (Abii *et al.*, 2011; Sobukola *et al.*, 2010). The major concern with the uptake of these contaminants by plants is their presence in plant produce consumed by humans. In countries such as Bangladesh, China, Hungary and India, Arsenic (As) is found at high concentration in ground water and surface soil (Chen *et al.*, 2006). Excessive uptake of As by crop plants may present a food safety problem. This exemplified by recent findings that rice (*Oryza sativa*) is particularly efficient in As uptake from paddy soil, leading to accumulation in rice grain at concentrations that may pose a health risk to people consuming large amounts of rice in their diet (Williams *et al.*, 2007; Zhu *et al.*, 2008). Similarly, Lead (Pb) also causes a serious problem as the levels of Pb in soil that are toxic to plant are not easy to evaluate. However, it is generally agreed that soil Pb concentrations ranging from 100 to 500 ppm are considered to be excessive (Pendias and Pendias, 1984).

The Root-knot nematode *Meloidogyne graminicola* has emerged as a serious pest of rice in most rice growing region of the world (Gaur & Pankaj, 2011). In India the nematodes are known to cause damage especially in rice nurseries and upland rice crops in the eastern and southern coastal region but are now prevalent in most rice growing regions of the country. In the north Indian Indo-Gangetic plains Gaur *et al.*, 1993 reported two species of root-knot nematodes, *viz.* *M. graminicola* and *M. triticooryzae* infesting both wheat and rice crops grown in succession in the rice-wheat cropping systems. Rice is not only a staple food but also commercially important; especially in the basmati rice which is a premium product having high market demand in India as well as for export. Therefore, quantities of produce as well as quality are important factors determining production and nutritional and commercial value. It is well known that the symptoms of nematode damage appear as chlorosis and stunted growth, poor tillering, inflorescence and grain production (Swain and Prasad, 1988). It has been demonstrated that these symptoms are result of reduced uptake of water and nutrients by the plant roots structurally modified and damaged by the nematode (Patnaik and Padhi, 1987). Histological studies have shown disruption of the xylem tissues in *Meloidogyne* galls as well as the presence of abnormally formed xylem vessels causing interruption of induced root water transport (Kirkpatrick *et al.*, 1991) and consequently the translocation of absorbed

nutrients to the shoot. Patil *et al.* (2013) have shown reduction in uptake of macro nutrients, Nitrogen (N), Phosphorus (P), Potassium (K) and micro nutrients, Iron (Fe) and Zinc (Zn) due to *M. graminicola* infection of the roots.

No reliable information is available on the effect of root-knot nematodes *M. graminicola* on uptake of heavy metals by the rice plants. The present studies were carried out to understand whether the uptake of heavy metals would follow same trend as reduced uptake of micro and macro nutrients due to increased nematode infestation levels or not. An investigation has been carried out on the effect of *M. graminicola* on the uptake of N, P, and K and heavy metals and plant growth of rice in field.

Materials and Methods

The effects of a serious of infestation levels of *M. graminicola* on the growth of rice and uptake of macronutrients, Nitrogen (N), Phosphorous (P) and Potassium (K) and heavy metals, Arsenic (As) and Lead (Pb) have been studied under field condition.

Two field plots about 500 m distance, known to be infested with *M. graminicola* at varying levels of infestation in several patches in the previous rice season was selected at IARI research farm, New Delhi. Seedlings of the rice cv. Pusa Basmati 1121 were raised in root-knot free nursery beds. One month old seedlings were transplanted in the field. After 4 weeks of transplanting, when foliar symptoms of yellowing and stunting became apparent, spots in the field were selected with varying levels of infestation, *viz.* no infection (I_0), low infection (I_1), medium infection (I_2), high infection (I_3). The degree of infestation was also verified by observing intensity of root galling in carefully removed seedlings from each spot. Four spots were marked for each level of infestation. In field, NPK fertilizers were applied with recommended dose of 150-50-50 kg/ha. Full dose of Phosphorus (P) and Potash (K) and half of the Nitrogen (N) was applied at the time of land preparation. The remaining Nitrogen was applied in two splits; one after 35 days of transplanting and other before flowering.

For analysis of nutrients in the plant samples, total Nitrogen content in plant was estimated in 1g dried samples by Kjeldhal method (Jackson, 1973). For Phosphorus and Potassium analysis, 1g dried plant samples were digested with nitric and perchloric acids. Phosphorous was assessed by Spectrophotometer (model UV

1800) using vanadate-molybdate reagent (Olsen *et al.*, 1954). Available potassium was determined by using Flame Photometer (model ME 881). Heavy metals, As and Pb content in the plant samples were determined by Atomic Absorption Spectrophotometer (model UV 1800).

The growth of plants was recorded on 110 days old plants in field experiments. The plant height was measured with help of a scale taking ten randomly selected plants at the time of maturity. The plants after harvesting were cut into shoot and root portion separately for determining dry weights. Shoot and root portions were washed thoroughly with water for removing soil particles and other materials and kept in dry oven at 65°C for 72 h were recorded using electronic weigh machine. Number of galls on roots per plant root system was also counted.

Result

The N content, on dry weight basis, in shoot of rice grown in nematode free soil (traces) was 0.51-0.50% and significantly reduced to 0.30-0.32% at high infestation level in plot 1 & plot 2, respectively. The main effect of *M. graminicola* infestation in reducing N content of shoot was significant (0.05P) in both plots. Similarly, the P content in healthy plants was 0.17% in plot 1 and 0.19% in plot 2 and reduced to 0.08% and 0.10% at high nematode infestation level. Averaged over all the nematode infestation levels, the P content was decreased from 0.17% to 0.08 (-53%) in plot 1 and 0.19% to 0.10 (47.3%) in plot 2, respectively, at high nematode infestation level (Table: 4.4.1, Fig: 4.4.1). The main effect of *M. graminicola* infestation in reducing P content of shoot was significant (0.05P) in plot 2 and non-significant effect was observed in plot 1. The K content in the rice shoot was 1.08%-1.08% in nematode free control; whereas, it was just 0.59%-0.61% at high infestation level. The main effect of *M. graminicola* infestation in reducing K content of shoot was significant (0.05P) in both plots. The concentration of all the three elements was negatively correlated to nematode infestation levels in the field (Table: 4.4.1, Fig: 4.4.1).

Table: 4.4.1. Effect of varying population densities of root-knot nematode, *Meloidogyne graminicola* on macro-nutrients uptake of rice cv. Pusa 1121 (shoot) in field

Nematode infestation levels	Macro nutrient content (%) (110 DAT)					
	PLOT 1			PLOT 2		
	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Nitrogen (N)	Phosphorus (P)	Potassium (K)
Traces	0.51	0.17	1.08	0.50	0.19	1.08
Low	0.50	0.16	0.84	0.42	0.13	0.83
Medium	0.38	0.13	0.77	0.36	0.12	0.70
High	0.30	0.08	0.59	0.32	0.10	0.61
Mean	0.42	0.13	0.8	0.39	0.13	0.8
CD 0.05 P	0.08	NS	0.19	0.04	0.06	0.16

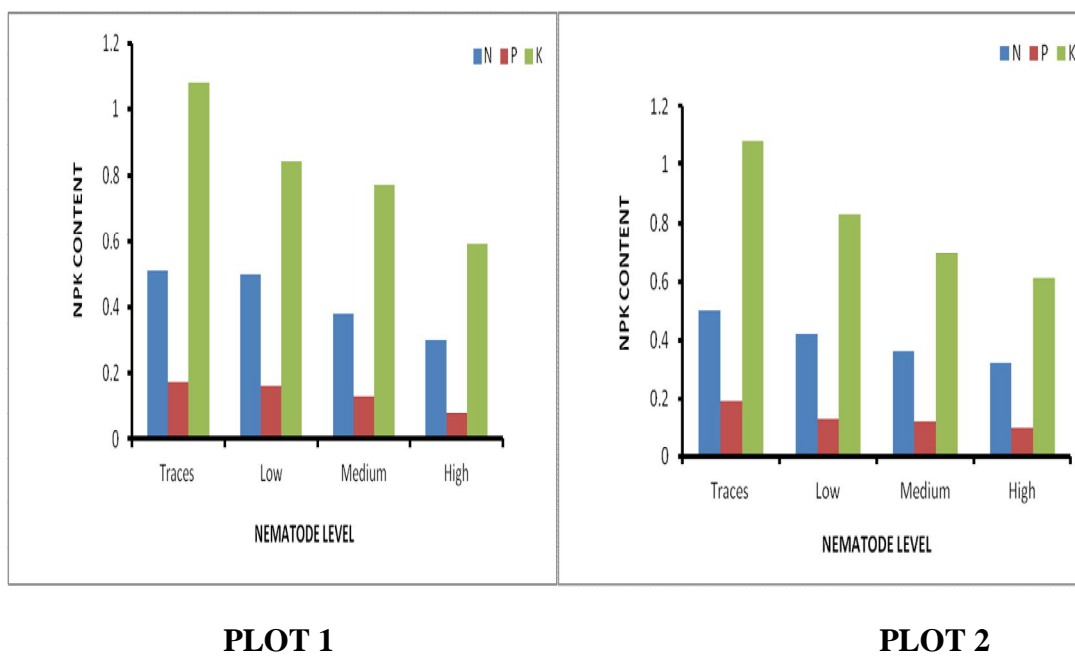


Fig 4.4.1 Effect of varying population densities of root-knot nematode, *Meloidogyne graminicola* on macro-nutrients content (%) of rice cv. Pusa 1121 (shoot) in field

Since the focus of these investigations was more on heavy metals uptake, its content was studied in root and shoot at 110 days after transplanting. The heavy metals, Arsenic (As) and Lead (Pb) was detected in the root, shoot and grains of rice plants. In case of As, its content was 0.05ppm in root and 0.02-0.03ppm in shoot respectively, in both the plots, in the case of nematode free plants. In the case of *M. graminicola* medium level of infestation, these contents increased to 0.06 and 0.11ppm in root and 0.07 and 0.09ppm in shoot in both plots, respectively. The increase in *M. graminicola* infestation to high level resulted in further increased in As content, both in root and shoot.

In case of As content, in root was 0.04 ppm in the nematode free control but it was significantly increased to 0.14 ppm at high infestation level in plot 1. The same trend was also observed in plot 2, as root contained 0.05 ppm As concentration in control plants whereas significant increase in the high infestation level of root contain 0.12 ppm concentration of As. In shoot, As concentration was low 0.02 ppm in the control plant whereas, plants with high infestation level had significantly higher As concentration 0.11ppm in plot 1. Similar trend was also observed in plot 2, with increasing concentration of As from control plant, 0.03 ppm to high nematode infestation plant 0.13 ppm (Table: 4.4.2, Fig: 4.4.2).

The concentration of As in grains was also found significantly higher in nematode infected plants. It was increased from 0.02-0.04 ppm in healthy plants to 0.08-0.09 ppm at high infestation levels in plots 1&2. The heavy metal uptake by plants was positively correlated to the nematode infestation level and significantly differed in control plants compared to high nematode infestation levels in plot 1 and non significant difference in plot 2. Similar trend was also seen in Lead (Pb) uptake by root. The 0.09 and 0.07 ppm concentration in control plant; was significantly increased to 0.28 and 0.22 ppm concentration at high nematode infestation level in plots 1 and 2. In case of shoot, the concentration of Pb was high in the high nematode infestation 0.28 and 0.16 ppm as compared to low concentration in control plant, 0.07 and 0.04 ppm in plots 1 and 2. The concentration of Pb in grains was found low at 0.03-0.04 ppm in the control plants whereas, it was higher in the high nematode infestation level at 0.09 and 0.11ppm in plot 1 and 2. The heavy metal uptake by plants was positively correlated to the nematode infestation level and significantly differed between control plants and high nematode infestation levels in plot 1 and

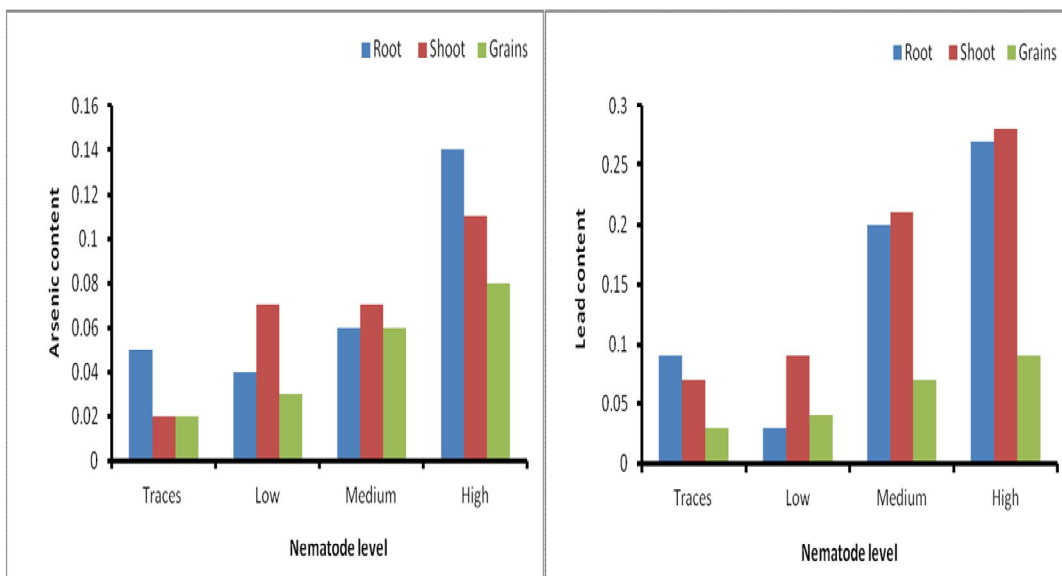
Table: 4.4.2. Effect of varying population densities of root-knot nematode, *Meloidogyne graminicola* on heavy metals uptake of rice cv. Pusa 1121 in field

PLOT 1

Nematode infestation levels	Arsenic (As) (ppm)			Lead (Pb) (ppm)		
	Root	Shoot	Grains	Root	Shoot	Grains
Traces	0.05	0.02	0.02	0.09	0.07	0.03
Low	0.04	0.07	0.03	0.03	0.09	0.04
Medium	0.06	0.07	0.06	0.20	0.21	0.07
High	0.14	0.11	0.08	0.27	0.28	0.09
Mean	0.07	0.06	0.05	0.16	0.16	0.06
CD 0.05 P	0.03	0.03	0.04	0.06	0.12	0.03

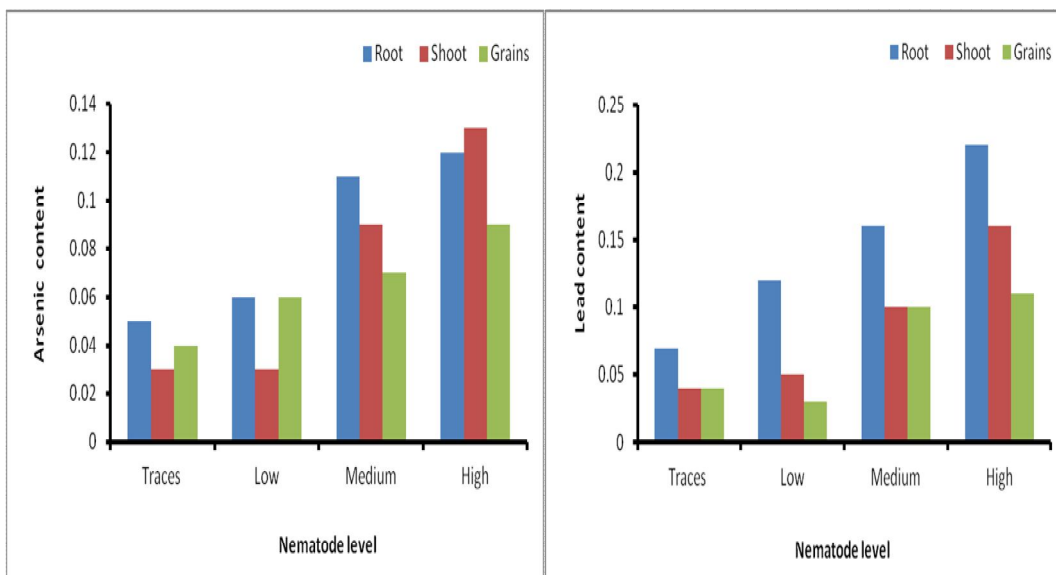
PLOT 2

Nematode infestation levels	Arsenic (As) (ppm)			Lead (Pb) (ppm)		
	Root	Shoot	Grains	Root	Shoot	Grains
Traces	0.05	0.03	0.04	0.07	0.04	0.04
Low	0.06	0.03	0.06	0.12	0.05	0.03
Medium	0.11	0.09	0.07	0.16	0.10	0.10
High	0.12	0.13	0.09	0.22	0.16	0.11
Mean	0.08	0.07	0.06	0.14	0.08	0.08
CD 0.05 P	0.04	0.05	NS	0.08	0.07	NS



PLOT 1

Fig 4.4.2 Effect of varying population densities of root-knot nematode, *Meloidogyne graminicola* on heavy metals uptake of rice cv. Pusa 1121 in field (Arsenic and Lead content in ppm)



PLOT 2

Fig 4.4.3 Effect of varying population densities of root-knot nematode, *Meloidogyne graminicola* on heavy metals uptake of rice cv. Pusa 1121 in field (Arsenic and Lead content in ppm)

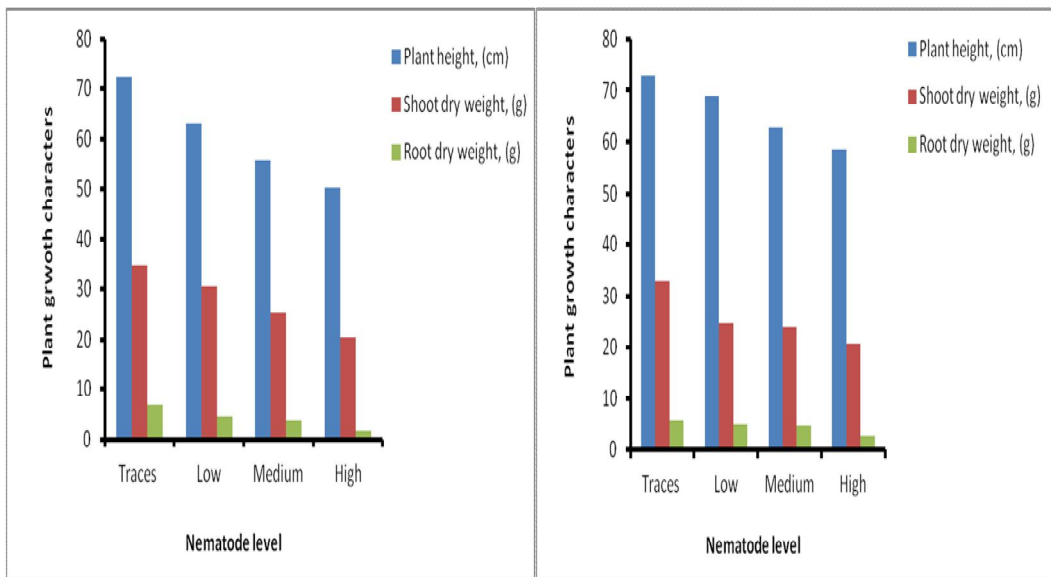
non significant difference in plot 2. The heavy metals, As and Pb concentration in rice generally followed a pattern of root > shoot > grains (Fig: 4.4.3).

The plant growth parameters like, plant height, shoot dry weight and root dry weight were recorded. Plant height was measured at 110 days after transplanting. The plant height was 72.3-72.8 cm of healthy plants; it was significantly reduced at the high infestation level 50.3-58.6 cm in plot 1 and 2. The shoot dry weight was also significantly reduced from 34.66-32.75g to 20.48-20.81g at high nematode infestation level in plot 1 and 2. More or less similar trend was observed in root dry weight of healthy plant was 6.76-5.85g reduced significantly to 1.80-2.78 g at high nematode infestation level (Table: 4.4.3, Fig: 4.4.4). The plant height, shoot dry weight and root dry weight were seen strong negative correlation to the nematode infestation level. Galls count in rice roots from different nematode infestation levels were recorded and significantly increased at high inoculum level than lower infested plants. There were also differences in size and shape of galls.

Table: 4.4.3. Effect of varying population densities of root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 1121 in field

Nematode infestation levels	PLOT 1				PLOT 2			
	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Gall index*	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Gall index*
Traces	72.30	34.66	6.76	0.5	72.80	32.75	5.85	0.5
Low	62.87	30.47	4.56	2.0	68.81	24.75	4.93	2.1
Medium	55.57	25.18	3.75	3.5	62.62	24.01	4.64	3.6
High	50.30	20.48	1.80	4.5	58.60	20.81	2.78	4.5
Mean	60.26	27.69	4.21	-	65.70	25.58	4.55	-
CD 0.05 P	5.03	4.42	1.35	-	6.19	6.45	1.49	-

*Gall index using 0-5 scale



PLOT 1

PLOT 2

Fig 4.4.4. Effect of varying population densities of root-knot nematode, *Meloidogyne graminicola* on plant growth of rice cv. Pusa 1121 in field

Discussion

The present study shows that the content of N, P and K in rice shoot was significantly reduced and was negatively correlated to the nematode infestation level. The plant height, shoot dry weight and root dry weight also decreased with increasing nematode infestation levels in field. However, concentration of heavy metals, As and Pb in shoots, roots and grains of rice was significantly increased and was positively correlated to the nematode infestation levels in the field. These results indicate that despite the increased heavy metal concentration, the concentration of nutrients was reduced in plants and their growth was suppressed by nematode infestation.

Miller and Cramer (2004) reported that the reduced N uptake primarily due to decreased fine root growth caused by the presence of the sedentary nematode. The reduced uptake of nutrients was not directly linked to a fine root reduction. Several histological reports showed that in roots infected by *Meloidogyne* there is an obstruction of the xylem as well as formation of non functional vessels in the surrounding areas of the galls and giant cells (Dorhout *et al.*, 1991; Kirkpatrick *et al.*, 1991). Thus, nutrient transport could be affected by a decrease of water transport. Dorhout *et al.* (1988) used the dye Tinopal CBS to study water movement in the xylem of tomato roots infected with *M. incognita*. They observed that the dye leaked out of the xylem vessels close to the giant cells, remaining in the apoplast of the galls. They also observed that the water did not flow from the cortex to the stele along the nematode body, or from the stele to the cortex. Therefore, nutrients would also be retained in the roots. The data on the influence of nematodes on N uptake by plants are controversial. Some reports detected an increase of N in the roots but no alteration in the shoots (Hunter, 1958; Shafiee & Jenkins, 1963) while others showed that there was no alteration in the roots and shoots (Melakeberhan *et al.*, 1985; Goncalves *et al.*, 1995). However, none of these reports dealt with nutrients absorption along with heavy metals were carried out experiments as done here.

The concentration of heavy metals, As and Pb significantly increased in shoots, roots and grains from nematode uninfected plants to high infestation levels and also had a positive correlation with increasing nematode infestation levels. Arsenite (As (III)) is the dominant As species in reducing environments such as flooded paddy soils (Marin *et al.*, 1993; Takahashi *et al.*, 2004; Xu *et al.*, 2008). The speciation of arsenic in the soil environment is dynamic. It can be biotically and abiotically inter-converted between the dominant solution phase inorganic species of

arsenate and arsenite, the oxidized and reduced forms respectively. Both of them can be taken up by rice roots (Abedin *et al.*, 2002). Iron plaque is commonly formed on the surfaces of roots of aquatic plants including rice by releasing oxygen to their rhizosphere through aerenchyma, resulting in the oxidation of ferrous iron to ferric iron, and the precipitation iron oxide on the root surface (Armstrong, 1964). The presence of iron plaque can sequester As and form a buffer zone that alters the entry of As into plants (Liu *et al.*, 2004). The presence of iron plaque can potentially alter the traditionally perceived phosphate-arsenate competition.

Mycorrhizal fungi and other microorganisms in the rhizosphere benefit the rice plants. There are many reports about effects of mycorrhizal fungi on As acquisition by plants. The fungi could restrict As translocation from roots to shoots although the mechanism is not clear (Liu *et al.*, 2005; Chen *et al.*, 2007). Other bacteria, such as Ammonia-Oxidizing Archaea (AOA), Bacteria (AOB), iron reducing bacteria, sulphate reducing prokaryotes, are abundant in paddy soils (Chen *et al.*, 2008; Wang *et al.*, 2009; Liu *et al.*, 2009; Chen *et al.*, 2008). Some of them involved in iron reduction or oxidation in paddy soil probably cause transformation of As species, leading to As co-precipitation with, or adsorption to, Fe (III) minerals in soil. Our results indicated that As uptake is increased from root to shoot because damaged root cortical cells by *M. graminicola* and galls acts as a nutrient sink, diverting nutrients to its giant cells may predisposed the large number of accumulated As species in reduced state under anaerobic condition near the root zone. Thermodynamically, reduction of arsenate to arsenite can occur quite readily at intermediate redox potentials (Inskeep *et al.*, 2002). Flooding of paddy soils leads to mobilization of arsenite into the soil solution and enhanced As bioavailability to rice plants (Xu *et al.*, 2008). This is the reason why South East Asian countries like Bangladesh and West Bengal in India are severely affected by arsenic contamination in rice cultivation because of growing rice under submerged condition. Soil type is another factor, clay soil majorly consist of these region may retain the water well compared to Delhi region having alluvial soil retains less water due to increased porosity, so that As speciation cannot remained under the redox status.

Arsenic has a low mobility with respect to translocation from roots to shoots in rice. Arsenite appears to be the main As species that is transported from the root cortical cells to xylem vessels because in all of the plant species studied, arsenite is

the predominant form of As in the xylum sap, accounting for 60-100% of total As. It is generally believed that As concentrations decrease in the order of roots > stems and leaves > husks > grain (Abedin *et al.*, 2002; Zhao *et al.*, 2009; Liu *et al.*, 2006). The high concentration of As in the husk is probably derived from the xylematic transport of As. Arsenite was translocated from root to shoot probably up to husk. By contrast, accumulation of As in the grain occur primarily through phloem. Our results, that As uptake was decreased from root > shoot > grains, was in conformity with the above cited reports that As species is transported from the root cortical cells to xylem vessels and disturbing xylem vessels by *M. graminicola* led to increased As translocation to grains via phloem cells.

Pb uptake studies in plants have demonstrated that roots have an ability to take up significant quantities of Pb whilst simultaneously greatly restricting its translocation to above ground parts (Lane and Martin, 1977). Our result was in agreement with above report that Pb uptake decreased from root to shoot and grains. This corroborates with Miller and Koeppe (1971) findings who demonstrated that Maize (*Zea mays* L.) plants could translocate and accumulate significant quantities of Pb in the leaves in a concentration dependent manner.

Pb moves predominantly in the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of Pb between root and shoot. This may in part account for the reports of higher accumulation of Pb in roots compared to shoots (Jones *et al.*, 1973). As *M. graminicola* advanced in cortical cells and disturbing the xylem vessels and water translocation, the movement of Pb got localized in roots. The limiter transport of Pb from roots to other organs is due to the barrier of the root endodermis. It appears that casparian strips of the endodermis are the major limiting factor restricting Pb transport across endodermis in the central cylinder tissue (Seregin and Ivanov, 1997). According to Lane and Martin (1977) the endodermis appears to act as a partial barrier since some of the Pb moves up through the vascular tissues and diffuse out into the surrounding tissues. This provides evidence that Pb moves into the symplast. That movement of Pb in the root is primarily via the apoplast is also supported by the report that large proportion of Pb is readily extractable in water (Broyer *et al.*, 1972). The possibility of symplastic transport of Pb has been demonstrated in onion roots and garden cress hypocotyls (Wierzbicka,

1987). The content of Pb in various plant organs tends to decrease in the following order: roots > leaves > stem > inflorescence > seeds. However this order can vary with plant species (Antosiewicz, 1992). In onion plants, absorbed Pb is localised in highest concentration in the root tips followed by proximal parts of the root, while its lowest concentration is found in the root base (Michalak and Wierzbicka, 1998). Our result was in agreement with above report that content of Pb localized in roots are higher concentration and decreased in order to shoot and grains.

As this work may be the first attempt to explore the knowledge about the nematodes and heavy metals interaction and mechanism of uptake of heavy metals by the plant via nematode parasitism. Many reports are on direct relationship between plants and heavy metals; this is the one which stated the triangle relation between plants, heavy metals and plant parasitic nematodes parasitism. The future issues are which genes and enzymes responsible for increased heavy metals uptake in plants from the nematode point of view is needed.

Crop production is one of the most important components of agriculture that provides food, fibre, fuel and various other products necessary for sustaining mankind and animals required for various purposes. Productivity of crops depends not only on their genetic potential but also on numerous environmental factors. Many biotic and abiotic stresses are responsible for limiting crop production. Such factors are also known to affect the nutritive value, consumer safety and other commercially important attributes of the produce besides the realized yield.

Among the most important food crops, rice occupies the prime position being the staple diet for over 2.7 billion people and forming significant component of the food of many others around the globe. The rice crops also suffer from the attacks of large number of pests and pathogens. Plant parasitic nematodes are now considered significant pests of various crops including rice and their potential to cause huge crop losses in the forms of reduced yield by themselves as well as through complexes with other pathogens has been demonstrated in numerous studies conducted in many countries.

The root-knot nematode, *Meloidogyne graminicola* (Golden and Birschfield, 1965), is a pest of international importance to rice crop around the world (Gaur and Pankaj, 2011; Dutta *et al.*, 2012). It is known to cause severe reduction in rice yields in most upland and irrigated paddies as well as to some extent in the lowland and deep water ecosystems. It is known to infect and cause serious damage to cereals, especially rice, in many countries causing estimated yield losses of between 20% and 80% (Arayarungsarit, 1987; Bridge and Page, 1982; Netscher and Elan, 1993; Soriano Reversat, 2003; Padgham *et al.*, 2004 and Pokharel *et al.*, 2007). *M. graminicola* causes economic losses in rice that vary depending on the agroecosystem in which the rice crop is grown. Losses of 20% have been documented for rice cultivars IR 29 and IR 74 under intermittent flooding (Plowright, 1990), 30% in semi deep water rice (Prasad *et al.*, 1990), and of 70% under saturated conditions when the nematode infects young susceptible seedlings at transplanting (Prot *et al.*, 1994). Jairajpuri and Baqri, 1991 reported grain yield losses from 16 to 32%.

When it comes to nutrition or other commercial attributes the quality of produce is as important as the quantity. Various studies have shown reduction in the uptake of macro- and micronutrients and water by the nematode affected roots and other tissues of the plants which explain much of the reduced plant growth. Nematodes can also enhance the rates of catabolic processes like respiration and decrease the rates of anabolic phenomena including photosynthesis by the plants. Decreased uptake of vitally important macronutrients, i.e. NPK and important micronutrients such as Iron and Zinc has also been reported (Patil, 2011; Patil *et al.*, 2013).

The increasing awareness to health hazards due to high contents of toxic heavy metals in food has become a serious issue. The soils and water bodies in some regions naturally have high levels of these heavy metals in forms available to plants thus, increasing their levels in the plants produce as well as in the primary consumers, i.e. herbivorous, animals, and finally, the entire food chain.

Arsenic levels are high in West Bengal and nearby eastern India and other areas. The heavy soils in these regions and the low soil oxygen levels in the rice ecologies lead to higher levels of Arsenite, the reduced forms of Arsenic oxides. The Arsenite is more toxic compared to Arsenate. It causes various kinds of skin disorders and other health hazard and also lethal at higher levels. Increasing levels of heavy metals due to soil pollution from industrial effluents is also a matter of serious concern. It not known whether the nematode infection on roots of rice would result in decreased uptake of heavy metals on the same pattern as the micro- and macronutrients. This could answer the question whether the nematode infection of the plant would increase or decrease the health hazards due to uptake of such matters and their accumulation in the consumable parts of the crops. The review of literature did not reveal any direct information that could throw light on these important aspects. Hence, the present investigations were undertaken which appears to be the first planned study on this aspect.

Studies were conducted on the arsenic contaminated clay loam soil of West Bengal. The results indicated higher content of arsenic in the root and shoot of rice field plant grown in soil inoculated with *M. graminicola* compared to the plants grown in the same soil without nematode inoculation. This was a trend directly contrary to the one reported for the effects of the same nematode on uptake of macro- and micronutrients, as has been reviewed in the preceding Chapter 2. More studies

were conducted on the sandy loam soil of Delhi artificially contaminated with different levels of arsenic, cadmium and lead and different population densities of *M. graminicola*. A field trial was also carried out at two sites at the IARI farm which received the waste water from city drains having high levels of Arsenic and Lead. In all the above studies, a common trend was observed that the infection on roots of rice plants by root-knot nematode, *M. graminicola* caused significant reduction in the NPK content of root and shoots of the rice plants but higher levels of arsenic, lead and cadmium were found in such plants compared to the nematode free plants. These levels were higher at higher contamination levels of the heavy metals and at higher population densities of *M. graminicola*, although not in the arithmetic proportion.

The growth of the rice plants in terms of plant height, dry weights of shoots and roots was marginally reduced at the higher concentration of arsenic, lead and cadmium compared to their lower concentration. The root galling was not significantly affected due to the three elements of heavy metals. This could show that the nematode infection of roots may not only decrease the quantity of produce and its nutritive value but also increase the possible health hazards due to higher contents of toxic heavy metals. As would be expected, the content of heavy metals was relatively lower in the shoot and further in grain compared to the roots. Hence, the content was lower in plants parts in some proportion to the distance and tissue types from the sites of absorption that is root. The results of different experiments have been discussed in the preceding Chapters 4.1-4.

There have been some earlier studies explaining the possibilities of greater uptake of the arsenic and other heavy metals compared to that of macro- and micronutrients (Abedin *et al.*, 2002; Liu *et al.*, 2004). The present findings have highlighted the necessity of further research on this important area to understand the reasons for the differential uptake of the heavy metals compared to the macro-nutrients uptake, as well as to find ways and means to reduce or avoid the uptake of toxic heavy metal especially when the fields are infested with nematodes such as *M. graminicola*. These findings have also added another dimension to the possible qualitative damage to the food crops and enhancement in health hazard in addition to the quantity, product quality and nutritional values, which are important not only to the farmers but also to the general public and consumers across the food chain.

The plant parasitic nematodes get their food from the cells of the root or shoot of plants infected by them. In the process they injure the cells causing death or deformation. Some nematodes, like the root-knot nematodes infect the fresh root tissues and have intricate host-parasite relationships that involve biochemical interactions between nematode saliva and plant amino acids. Best known is the excessive conversion of tryptophan to indole-acetic acid, leading to giant cell and gall formation due to hypertrophy and hyperplasia of some of the root tissues. This creates a nutritional and metabolic sink for the nematodes, but interferes with the uptake and translocation of nutrients and water to other plant parts. Much is known about reduction of growth and yield but too little about the effect of root knot nematodes on uptake of macro- and micronutrients by the plants, growth of plants and possible health hazards due to high contents of toxic heavy metals in the agroecological systems where the nematodes play important role, whether they enhance or decrease the uptake of heavy metals by the plants becomes a serious issue. Investigations have been carried out to address these issues using the root-knot nematode, *Meloidogyne graminicola* infecting rice as a model in experiments carried out at IARI, New Delhi.

A preliminary study was done to see the effect of *M. graminicola* inoculation @ $4 \text{ J}_2 \text{ cm}^{-3}$ on the As content of root and shoot of Rice cv. Pusa 1121 grown in clay loam soil containing 12 and 20 ppm As, obtained from BCKV, Kalyani. The results indicated higher content of arsenic in the root and shoot of rice field plant grown in soil inoculated with *M. graminicola* compared to the plants grown in the same soil without nematode inoculation.

Further studies were conducted on the sandy loam soil of Delhi artificially contaminated with different levels of arsenic, cadmium and lead. The effects of two inoculum levels of *M. graminicola* on the uptake of macronutrients, Nitrogen (N), Phosphorus (P) and Potassium (K) and heavy metal, Arsenic, Lead and Cadmium and plant growth of rice were studied under pot culture. Here the trends were contrary to those reported in literature for the effects of the same nematode on uptake of macro- and micronutrients. The effect of *M. graminicola* inoculum was apparent in the reduced plant growth of rice measured in terms of shoot height and dry weights of

root and shoot. The number of galls on the root system was significantly higher though not in the same arithmetic ratio at the inoculum level of $8 \text{ J}_2 \text{ cm}^{-3}$ compared to the lower level of $2 \text{ J}_2 \text{ cm}^{-3}$ soil.

A field trial was also carried out at two sites at the IARI farm which received the waste water from city drains having high levels of Arsenic and Lead. The heavy metal Arsenic uptake by plants was positively correlated to the nematode infestation level and significantly differed in control plants compared to higher nematode infestation levels in plot 1 and nonsignificant difference in plot 2. Similar trend was also seen in Lead (Pb) uptake by root. The heavy metals, As and Pb concentration in rice generally followed a pattern of root > shoot > grains. The plant height, shoot dry weight and root dry weight showed strong negative correlation to the nematode infestation level. Root gall count in rice roots from different nematode infestation levels was recorded. These were significantly greater at the higher inoculum level.

This research identifies following salient points.

1. Nematodes affect the macronutrient and heavy metal uptake and transport from rhizosphere to root, shoot and grain.
2. Nematode infection may decrease the uptake of plant nutrients but increase uptake of certain heavy metals.
3. The reduction in nutrient uptake and accumulation of heavy metals in the grains is correlated with the level of nematode infection.
4. The consumer interest would be affected adversely due to poor quality and food value and possibly higher levels of heavy metals in shoot and grain after the harvest of a nematode infested crop.

As this work may be the first attempt to explore the nematode and heavy metals interaction, there is a need to understand the mechanism of uptake of heavy metals by the plant via nematode parasitism. There have been some earlier studies explaining the possibilities of greater uptake of the arsenic and other heavy metals compared to that of macro- and micronutrients independent of nematodes. The present findings have highlighted the necessity for further research on this important area to understand the reasons, for the differential uptake of the heavy metals compared to the macro- and micronutrient uptake. It is also necessary to find ways and means to reduce or avoid the uptake of toxic heavy metals especially when the

fields are infested with nematodes such as *M. graminicola*. These findings have also added another dimension to the nematode damage to crops in the form of increased health hazards to consumers besides the quantity, product quality and nutritional values.

ABSTRACT

Effect of Root-knot Nematode, *Meloidogyne graminicola* on the Nutrients and Heavy Metal Uptake and Growth of Rice, *Oryza sativa*

Investigations have been carried out on the effect of the root-knot nematode, *Meloidogyne graminicola*, a serious pest of rice, on the content of the major plant nutrients, viz., Nitrogen (N), Phosphorus (P) and Potassium (K) and the heavy metal, Arsenic (As), Lead (Pb) and Cadmium (Cd) and growth of the *basmati* rice, *Oryza sativa*, cv. Pusa 1121. The results show that the content of N, P and K in rice shoot were significantly reduced and were negatively correlated to the nematode inoculum level. The content of NPK after 45 and 110 days of transplanting was significantly lower in the shoot of rice inoculated with 2 and 8 J2 cm⁻³ soil compared to nematode-free plants, the reduction being more at the higher inoculum level. The plant height and shoot and root dry weights were also decreased with increasing nematode inoculum level. A field trial was also carried out at two sites at the IARI farm which received the waste water from city drains having high levels of Arsenic and Lead. The heavy metal Arsenic uptake by plants was positively correlated to the nematode infestation level. Similar trend was also seen in Lead (Pb) uptake by root. The heavy metals, As and Pb concentration in rice generally followed a pattern of root > shoot > grains. The plant height, shoot dry weight and root dry weight showed strong negative correlation to the nematode infestation level. Root-knot indices were significantly increased at high inoculum levels. The results have revealed differences in the uptake of plant nutrients versus heavy metals from soil infested with root-knot nematode, *M. graminicola* infecting rice roots. The results have indicated to possible increased health hazards to consumers besides the crop losses due to reduction in the quantity, product quality and nutritional values if plant parasitic nematodes are not managed.

Key words: Nitrogen, Phosphorus, Potassium, Arsenic, Lead, Cadmium, Heavy metal, Soil pollution, *Meloidogyne graminicola*, Nematode, Rice.

Qana, Aarayaja sara[vaa kl vaW tqa palak t%aa] dyaNa pr jaD,

gatz sat kRn, nayaDagayal gahalkaba ka pBaara

sar

baasantl Qana, Aarayaja sara[vaa, kRjajajait pbaa 1121 kl vaWtqa tqa ma%ya padp-
palak t%aa] yaqaa ma[Tajana 3e', fa'farsa 3pl' evalpaTiSayana 3k'evalBaarl QatuAa]yaqaa
Aasainak 3e esa', laD 3pl bal'evalMkDimayana 3sal DI' koANa]pr Qana koek gaa]r
pIDk jaD, -gatz sat kRn, nayaDagayal gahalkaba ka pBaara &at krna koilae Aoyaya ike
gae pirNaana dSaotohMik Qana koprah ma]na,pl evalMkoANa ma%apNa \$p saokma haogae tqa
vaosakRn-inavaSadya str sao?Na%ak \$pba sahsabM]at qaa. sat kRn saom]et pa]aMkl t]naa
ma] 2 evalM8 ja2 saom]-2 ma]la kosaqa inava]Sat Qana kopaa]Mkoprah ma] rapa[- ko45 evalM
110 idna baad, era,pl evalMkoANa ma%apNa \$p saokma pae gae Aat yah kmal]ccatr
inavaSadya str pr AiOak qal. sat kRn inavaSadya str ko baZnao kosaqa-saqa padp -}Baa[-
tqa prah evalMjaD, koSalik Baar]na]Bal kmal h]f-. BaaO k]D AO sa]qana p]xæ kodaosqal]pr
ek p]xæ Tajala Bal lagaaya gayaa jaa]ccastriya Aasainak evalM]aD ya]t Sahrl vaiht jala sao
is]at qaa pa]a]M]vaara Baarl Qatu Aasainak ka]dyaNa, sat kRn pkap str kosaqa Qana%ak
\$p saosahsabM]at qaa. jaD, d]vaara laD 3pl bal' ko]dyaNa ma]Bal yah] Z]ya d]Ka gayaa. Qana ma]l
Baarl QatuAa] e esa evalMpl bal sandta ka saam]atya ya] Z]ya d]Ka gayaa, jaD> prah >
danao Paadp -}Baa[-, prah Salik Baar evalM]aD, Salik Baar naosakRn pkap str kosaqa ek
d]Z, ?Na%ak sahsabM]a dSaaya.]cca inavaSadya str]pr jaD,-gatz sat kRn Gaataka]na]ma%apNa
\$p saoiOak baZatrl d]kl ga[-. pirNaana Qana kl jaD]Mkaos]at]inat krnao va]ao jaD, -gatz
sat kRn ma] gahalkaba ya]t ma]la sao padp palak t%aa]masasa Baarl QatuAa]Mko]dyaNa M]M
iBamataeMdSaotohM] yaid padp -prjalval [na sat kRn]ya]Mka p]a]na na ikya jae taopirNaana
dSaotohM]ik fsala kl gah]va-aa,]%ad-gah]va-aa evalMpa]Na-ma]na]M]M]kmal kosaqa-saqa [sasao
]pBa]ta ka svaasqya Bal salikT ma]p]Dnao kl sa]h]va]na hO

m#ya Sabd : maa[Tajana, f#farsa, paTiSayana, Asainak, laD ,kDinayana, Baarl Qartu malla-
p'dMaNa, malaayaDagaayanal galhanalKalaa Qaana

BIBLIOGRAPHY

- Abedin, M. J. & Meharg, A.A. (2002a).** Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). *Plant and Soil*, **243**: 57–66.
- Abedin, M. J., Cottepp-Howells, J. & Meharg, A.A. (2002b).** Arsenic uptake and accumulation in rice (*Oryza sativa* L.) irrigated with contaminated water. *Plant and Soil*, **240**: 311-319.
- Alam, M. M. and Saxena, S. K. (1975b).** The effect of *Tylenchorhynchus brassicae* on the water absorption capacity of roots. *Geobios*, **2**:161-162.
- Alam, M. M., Ali, Q. U., Masood, A. and Khan, A. M. (1975).** Studies on the changes induced by the infection of root-knot nematode, *Meloidogyne incognita* in tomato and the stunt nematode, *Tylenchorhynchus brassicae* in cabbage and cauliflower roots. *Indian Journal of Experimental Biology*, **14**:515-518.
- Alloway, B.J. (1995).** Heavy Metals in Soils. 2nd Ed. Blackie Academic & Professional, An imprint of Chapman & Hall, U K.
- Anonymous, (1989).** IRRI toward 2000 and beyond. Manila, Philippines: International Rice Research Institute. 71 pp.
- Anonymous, (2001).** National Research Council. *Arsenic in drinking water: 2001 Update*, National Research Council: Washington, DC, 2001.
- Arayarungsarit, L. (1987).** Yield ability of rice varieties in fields infested with root-knot nematode. *Int. Rice Res. Notes*, **12**: 14.
- Armstrong, W. (1964).** Oxygen diffusion from the roots of some British bog plants. *Nature*, **204**: 801–802.
- Arshad, M., Silvestre, J., Pinelli, E., Kallerhoff, J., Kaemmerer, M., Tarigo, A., Shahid, M., Guiresse, M., Pradere, P. & Dumat, C. (2008).** A field study of lead phytoextraction by various scented *Pelargonium* cultivars. *Chemosphere*, **71**: 2187–2192.
- Ashour, E.H., El-Mergawi, R.A. & Radwan, S.M.A. (2006).** Efficacy of *Pseudomonas* to Phyto remediate Nickel by Canola (*Brassica napus* L.). *Journal of Applied Sciences Research*, **2**:375-382.

- ATSDR, (2003).** Agency for Toxic Substances and Disease Registry. Taken from URL address: <http://www.atsdr.cdc.gov/> on July, 2013.
- Begum, S. (2007).** Effect of Arsenic on the development of Root-knot nematode *Meloidogyne incognita* in Roselle (*Hibiscus sabdariffa*). Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur.
- Bergeson, G. B. (1966).** Mobilisation of minerals to the infection site of root-knot nematode. *Phytopathology*, **56**:1287-1289.
- Blevins, D. G., Dropkin, V. H. & Ludders, V. D. (1995).** Macronutrient uptake, translocation and tissue concentration of soybeans infested with the soybean cyst nematode and elemental composition of cysts isolated from roots. *Journal of Plant Nutrition*, **18**:579-591.
- Bin, Xu. Ying., Sheng, Mai. Yao., JinTian, Lin. & Feng Zhi. Xin. (1999).** Physiological variation in groundnut plants infected with *Meloidogyne arenaria*. *Journal of Huazhong Agricultural University*, **18**:121-124.
- Bridge, J. Luc, M. & Plowright, R.A. (1990).** Nematode parasites of rice. In: Luc M, Sikora RA, Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture, CAB International, UK, pp. 69-108.
- Bridge, J. (1990).** Nematode parasites of rice. In: Luc M, Sikora RA, Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture, CAB International, UK, pp. 75-107.
- Carey, A.M. , Scheckel, K.G., Lombi, E., Newville, M. & Choi, Y. (2010).** Grain nloading of arsenic species in rice. *Plant Physiology*, **152**, 309–19.
- Carneiro, R. G., Mazzafera, P., Carlos, L., Ferraz, C. B., Muraoka, T. & Trivelin. P. C. O. (2002).** Uptake and translocation of nitrogen, phosphorus and calcium in soybean infected with *Meloidogyne incognita* and *M. javanica*. *Fitopatologia Brasileira*, **27**:34-38.
- Caroli, S., D'Ilio, S., Alessandrelli, M. & Cresti, R. (2002).** Arsenic content of various types of rice as determined by plasma-based techniques. *Microchemistry Journal*, **73**, 195–201.
- Caroli, S., Frazzoli, C., D'Amato, M. & Záray, Gy. (2007).** Arsenic and other potentially toxic trace elements in rice. In *The Determination of Chemical Elements in Food: Applications for Atomic and Mass Spectrometry*; Caroli, S., Ed.; John Wiley: Rome, Italy, 2007.

- Cecchi, M. , Dumat, C., Alric, A., Felix-Faure, B., Pradere, P. & Guiresse, M. (2008).** Multi-metal contamination of a calcic cambisol by fallout from a lead-recycling plant. *Geoderma*, **144**: 287–298.
- Chandel, S.T., Gaur, H.S. & Alam, M. M. (2002).** Population dynamics of the root-knot nematode, *Meloidogyne triticoryzae* under five rice-based cropping systems. *Arch. Phytopath. Pflanz*, **35**: 43-51.
- Chen, Z., Zhu, Y.G., Liu, W.J. & Meharg, A.A. (2005).** Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytologist*, **165**: 91–97.
- Chen, B.D., Xiao, X.Y., Zhu, Y.G., Smith, F.A., Xie, Z.M. & Smith, S.E. (2007).** The arbuscular mycorrhizal fungus *Glomus mosseae* gives contradictory effects on phosphorus and arsenic acquisition by *Medicago sativa* Linn. *Science of the Total Environment*, **379**: 226–234.
- Chen, X.P., Zhu, Y.G., He, J.Z. & Shen, J.P. (2008).** Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? *Environmental Microbiology*, **10**: 1978-1987.
- Chen, X.P., Zhu, Y.G., Hong, M. N., Kappler, A. & Xu, Y.X. (2008).** Effect of different forms of nitrogen fertilizers on arsenic uptake by rice plants. *Environmental Toxicology and Chemistry*, **27**: 881-887.
- Das, H.K., Mitra, A.K., Sengupta, P.K., Hossain, A., Islam, F. & Rabbani, G.H. (2004).** Arsenic concentration in rice, vegetables and fish in Bangladesh: A preliminary study. *Environment International*, **30**: 383–387.
- Davies, B.E. (1995).** Lead, *In: Heavy Metals in Soils*, B.J. Alloway (Ed.), Blackie Academic, London, pp. 206-223.
- Dwivedi, S., Mishra, A., Tripathi, P., Dave, R., Kumar, A., Srivastava, S., Chakrabarty, D., Trivedi, P. K., Adhikari, B., Norton, G. J., Deo, R. D., Tripathi., & Nautiyal, C.S. (2012).** Arsenic affects essential and non-essential amino acids differentially in rice grains: Inadequacy of amino acids in rice based diet. *Environment International*, **46**: 16–22.
- Dorhout, R., Kolloffel, C. & Gommers, F.J. (1988).** Transport of an apoplastic fluorescent dye to feeding sites induced in tomato roots by *Meloidogyne incognita*. *Phytopathology*, **7**: 1421-1424.

- Dorhout, R., Gommers, F.J. & Kolloffel, C. (1991).** Water transport through tomato roots infected with *Meloidogyne incognita*. *Phytopathology*, **81**: 379-385.
- Dos Santos, J. M. & Ferraz, S. (1981).** Effect of *Meloidogyne exigua* on the absorption and translocation of nutrients in coffee plants. *Fitopatologia Brasileira*, **6**:333-340.
- Dutta, T. K. Ganguly, A. K. & Gaur, H. S. (2012).** Global status of rice root-knot nematode, *Meloidogyne graminicola*. *African Journal of Microbiology Research*, **6**: 6016-6021.
- Duxbury, J.M. , Mayer, A.B., Lauren, J.G. & Hassan, N. (2003).** Food chain aspects of arsenic contamination in Bangladesh: Effects on quality and productivity of rice. *Journal of Environment Science Health*, **38**: 61–69.
- Evans, K., Trudgill, D. L. & Brown, N. J. (1977).** Effects of potato cyst nematodes on potato plants vascular root system development in lightly and heavily infested susceptible and resistant varieties and its importance in nutrient and water uptake. *Nematologica*, **23**:153-164.
- Eriksson, J. E. (1989).** The influence of pH, soil type and time on adsorption and uptake by plants of Cd added to the soil. *Water, Air, and Soil Pollution*, **48**:317-335.
- Fazel, R., Ahmed, S., Alexander, I. J., Mwinyihija, M. & Killham, K. (2011).** Effect of Superphosphate and Arbuscular Mycorrhizal Fungus *Glomus mosseae* on Phosphorus and Arsenic Uptake in Lentil (*Lens culinaris* L.). *Water, Air, & Soil Pollution*, **221**: 169-182.
- Gad, N. & Ismail, A.E. (2011).** Suppressive effect of cobalt on sugar beet infested with *Meloidogyne arenaria* grown in newly reclaimed sand soils and its role on sugar beet production and quality. *Journal of Applied Sciences Research*, **7**: 1583-1590.
- Gaur, H.S. Khan, E. & Sehgal, M. (1993).** Occurrence of two species of root-knot nematodes infecting rice, wheat and monocot weeds in northern India. *Ann. Plant Protect. Sci*, **1**: 141-142.
- Gaur, H.S, Singh, J. Sharma, S.N. & Chandel, S.T. (1996).** Distribution and community analysis of plant-parasitic nematodes in rice-growing areas of Haryana, India. *Ann. Plant Protect. Sci*, **4**: 115-121.

- Golden, A. M. & Birschfield, W. (1965).** *Meloidogyne graminicola*, a new species of rootknot nematode from grass. *Proceeding of Helminthological Society Washington*, **32**:228-231.
- Goncalves, W., Mazzafera, P., Ferraz, L.C.C.B., Silvarolla, M. B. & Lima, M. M. A. (1995).** Biochemical basis of coffee tree resistance to *Meloidogyne incognita*. *Plantations Recherche Développement*, **2**: 54-58.
- Grover, P., Rekhadevi, P., Danadevi, K., Vuyyuri, S., Mahboob, M. & Rahman, M. (2010).** Genotoxicity evaluation in workers occupationally exposed to lead. *International Journal of Hygenic Environment Health*, **213**: 99–106.
- Gupta, D., Nicoloso, F., Schetinger, M. , Rossato, L., Pereira, L., Castro, G., Srivastava, S. & Tripathi, R. (2009a).** Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. *Journal of Hazard Mater*, **172**: 479–484.
- Gupta, D., Huang, H., Yang, X., Razafindrabe, B. & Inouhe, M. (2010b).** The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatins but the glutathione. *Journal of Hazard Mater*, **177**: 437–444.
- Haseeb, A., Srivastava, N. K. & Pandey, R. (1990).** The influence of *Meloidogyne incognita* on growth, physiology, nutrient concentration and alkaloid yield of *Hyoscyamus niger*. *Nematologia Mediterranea*, **18**:127-129.
- He, C.Q., Tan, G.E., Liang, X., Du, W., Chen, Y.L., Zhi, G.Y. & Zhu, Y. (2010).** Effect of Zn-tolerant Bacterial Strains on Growth and Zn Accumulation in *Orychophragmus Violaceus*. *Applied Soil Ecology*, **44**: 1-5.
- Hunter, A.H. (1958).** Nutrient absorption and translocation of phosphorus as influenced by the root knot nematode (*Meloidogyne incognita acrita*). *Soil Science*, **86**:245-250.
- Hurchanik, D. & Schmitt, D. P. (2003).** Relationship of *Meloidogyne konaensis* population densities to nutritional status of coffee roots and leaves. *Nematropica*, **33**:55-64.
- Hurchanik, D., Schmitt, D. P., Hue N. V. & Sipes, B. S. (2004).** Plant nutrient partitioning in coffee infected with *Meloidogyne konaensis*. *Journal of Nematology*, **36**:76–84.
- Inskeep, W.P., McDermott, T.R. & Fendorf, S. (2002).** Arsenic (V)/III cycling in soils and natural waters: chemical and microbiological processes. In:

Frankenberger JWT, ed. *Environmental Chemistry of Arsenic*. New York, NY, USA: Marcel, Dekker, 183-215.

- Jackson, M. L. (1973).** *Soil Chemical Analysis*. Prentice Hall of India Ltd., New Delhi.
- Jain, R.K. Mathur, K.N. & Singh, R.V. (2007).** Estimation of losses due to plant parasitic nematodes on different crops in India. *Ind. J. Nematol*, **37**: 219-220.
- Jairajpuri, M. S. & Baqri, Q.H. (1991).** Nematode pests of rice, Oxford and IBH Publisher, New Delhi, India, p. 66.
- Ji, W., Chenb, Z., Lia, D. & Nia, W. (2012).** Identifying the criteria of cadmium pollution in paddy soils based on a field survey. *Energy procedia*, **16**: 27-31.
- Jiang, X.J., Y.M. Luo, Q. Liu, S.L. Liu & Q.G. Zhao. (2004).** Effects of cadmium on nutrient uptake and translocation by Indian mustard. *Environmental and Geochemical Health*, **26**: 319-324.
- Kashem, M. A. & Singh, B.R. (2001).** Metal availability in contaminated soils: Effects of flooding and organic matter on changes in Eh, Ph and solubility of Cd, Ni and Zn. *Nutrient Cycling in Agro-ecosystems*, **61**: 247-255.
- Khan, M.R., Khan, S.M., Mohiddin, F.A. & Askary, T.H. (2006).** Effects of high nickel soil on root-knot nematode disease of tomato. *Nematropica*, **36**: 79-88.
- Kibria, M. G., Osman K.T. and Ahmed, M. J. (2006).** Cadmium and lead uptake by rice (*Oryza sativa* L.) grown in three different textured soils. *Soil & Environ*, **25**: 70-77.
- Kirkpatrick, T.L., Oosterhuis, D.M. & Wullschleger, S.D. (1991).** Interaction of *Meloidogyne incognita* and water stress in two cotton cultivars. *Journal of Nematology*, **23**: 462-467.
- Korcak, R.F. & Fanning, D.S. (1985).** Availability of applied metals as a function of type of soil material and metal source. *Soil Science*, **140**: 23-34.
- Krzyszowska, M. , Lenartowska, M. , Mellerowicz, E.J., Samardakiewicz, S. & Wozny, A. (2009).** Pectinous cell wall thickenings formation—a response of moss protonemata cells to lead. *Environmental Experiment Botony*, **65**:119–131.
- Kuffner, M., Puschenreiter, M., Wieshammer, G., Gorfer, M. & Sessitsch, A. (2008).** Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. *Plant Soil*, **304**: 35–44.

- Li, W.C., Ye, Z.H. & Wong, M.H. (2007).** Effects of bacteria on enhanced metal uptake of the Cd/Zn-hyper accumulating plant, *Sedum alfredii*. *Journal of Experimental Botany*, **58**: 4173–4182.
- Liu, M. C., Li, H. F. and Xia, L. J. (2000).** Differences of cadmium uptake by rice genotypes and relationship between the iron oxide plaque and cadmium uptake. *Acta Scientiae Circumstantiae*, **20**: 592-596.
- Liu, J., Li, K., Xu, J., Zhang, Z., Mac, T., Lu, X., Yang, J. & Zhu, Q. (2003).** Lead toxicity, uptake, and translocation in different rice cultivars. *Plant Science*, **165**: 793-802.
- Liu, Y., Zhu, Y.G., Chen, B.D., Christie, P. & Li, X.L. (2005).** Yield and arsenate uptake of arbuscular mycorrhizal tomato colonized by *Glomus mosseae* BEG167 in As spiked soil under glasshouse conditions. *Environment International*, **31**: 867–873.
- Liu, W.J., Zhu, Y.G., Hu, Y., Williams, P.N., Gault, A.G., Meharg, A.A., Charnock, J. M. & Smith, F.A. (2006).** Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza sativa* L.). *Environmental Science and Technology*, **40**: 5730–5736.
- Liu, X.Z., Zhang, L.M. , Prosser, J.I. & He, J.Z. (2009).** Abundance and community structure of sulfate reducing prokaryotes in a paddy soil of southern China under different fertilization regimes. *Soil Biology and Biochemistry*, **41**: 687-694.
- Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P. & Zhao, F.J. (2008).** Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *Proc. Natl. Acad. Sci. USA*. 105(29), 9931–9935.
- MacGowan, J.B. (1989).** Rice root-knot nematode *Meloidogyne graminicola* Golden and Birchfield 1965. Fla. Dept. of Agric. and consumer Serv., Div. Plant Ind., Nematology Circular No. 166, June 1989.
- Maestri, E., Marmiroli, M. Visioli, G. & Marmiroli, N. (2010).** Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environmental Experiment Botany*, **68**: 1-13.
- Mahalik, J. K. and Routaray, B. N. (2009).** Changes in chlorophyll and nitrogen content in blackgram mutant lines induced by different mutagenic treatments towards root-knot nematode infection. *Environment and Ecology*, **27**: 1997-2001.

- Maung, M. O. & Jenkins, W. R. (1959).** Effects of root-knot nematode *Meloidogyne incognita acrita* and a stubby-root nematode *Trichodorus christiei* on the nutrient status of tomato, *Lycopersicon esculentum*. *Plant Disease Reporter*, **43**:791-796.
- Melakeberhan, H., Webster, J.M. & Brooke, R.C. (1985).** Response of *Phaseolus vulgaris* to a single generation of *Meloidogyne incognita*. *Nematologica*, **31**: 191-202.
- Meon, S., Fischer, J. M. & Wallace, H. R. (1978).** Changes in free proline following infection of plants with either *Meloidogyne incognita* or *Agrobacterium tumefaciens*. *Physiological Plant Pathology*, **12**:251-256.
- Miller, A.J. & Cramer, M. (2004).** Root nitrogen acquisition and assimilation. *Plant and Soil*, **274**: 1-36.
- Miteva., Maneva., Hristova & Bojinova (2001).** Heavy Metal Accumulation in Virus-infected Tomatoes. *Journal of Phytopathology*, **149**:179-184.
- Moya, J.L., Ros, R. & Picazo, I. (1993).** Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. *Photosynthesis Research*, **36**:75-80.
- Netscher, C. & Erlan, X. (1993).** A root-knot nematode, *Meloidogyne graminicola*, parasitic on rice in Indonesia. *Afro Asian Journal of Nematology*, **3**:90-95.
- Obata, H. & Omebayashi, M. (1997).** Effects of cadmium on mineral nutrient concentrations in plants differing in tolerance for cadmium. *Journal of Plant Nutrition*, **20**: 97-105.
- Olsen, S., Cole, C., Watanabe, F. & Dean, L. (1954).** Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, D.C.
- Oteifa, B. A. (1952).** Potassium nutrition on the host in relation to infection by root-knot nematode, *Meloidogyne incognita*. Proceedings of the Helminthological Society of Washington **19**:99-104.
- Oteifa, B. A. & Elgindi, D. M. (1962).** Influence of parasitic duration of *Meloidogyne javanica* (Treub) on host nutrient uptake. *Nematologica*, **8**:216-220.
- Padgham, J.L. Duxbury, J.M. Mazid, A.M. Abawi, G.S. & Hossain, M. (2004).** Yield loss caused by *Meloidogyne graminicola* on lowland rainfed rice in Bangladesh. *J. Nematol*, **36**: 42-48.

- Padmaja, K., Prasad, D.D.K. & Prasad, A.R.K. (1990).** Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* seedlings by cadmium acetate. *Photosynthetica*, **24**: 399-405.
- Patil, J., Miller, A.J. & Gaur, H.S. (2013).** Effect of nitrogen supply form on the invasion of rice roots by the root-knot nematode, *Meloidogyne graminicola*. *Nematology*, **15**: 483-492.
- Patnaik, N.C. & Padhi, N.N. (1987).** Damage by rice root-knot nematode. *International Rice Research Newsletter*, **12**: 27.
- Plowright, R. and Bridge, J. (1990).** Effect of *Meloidogyne graminicola* (Nematoda) on the establishment, growth and yield of rice cv. IR36. *Nematology*, **36**: 81-89.
- Piotrowska, A., Bajguz, A., Godlewska-Zylkiewicz, B., Czerpak, R. & Kaminska, M. (2009).** Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). *Environmental Experiment Botany*, **66**: 507–513.
- Pizarro, I. & Gómez, M. (2003).** Evaluation of stability of arsenic species in rice. *Annals of Bioanalytical Chemistry*, **376**: 102–109.
- Pokharel, R.R. Abawi, G.S., Zhang, N. Duxbury, J.M. & Smart, C.D. (2007).** Characterization of Isolates of *Meloidogyne* from Rice-Wheat Production Fields in Nepal. *J. Nematol*, **39**: 221-230.
- Prasad, J.S, Panwar, M. S. & Rao, Y.S. (1985).** Occurrence of root knot-nematode, *Meloidogyne graminicola* in semideepwater rice. *Current Science*, **54**: 387-388.
- Prasad, J.S, Panwar, M. S. & Rao, Y.S. (1986).** Screening of some rice cultivars against the root-knot nematode *Meloidogyne graminicola*. *Ind. J. Nematol*, **16**: 112-113.
- Prasad, J. S., Panwar, M. S. & Rao, Y. S. (1990).** Influence of root-knot nematode infection on rice under simulated rainfed lowland conditions. *Nematologia Mediterranea*, **18**:195-197.
- Price, N. S., Clarkson, D. T. & Hauge, M. G. M. (1982).** The uptake of parasitism and phosphorus in oats influenced with the cereal cyst nematode, *Heterodera avenae*. *Revue de Nematologie*, **5**:321-325.
- Prot, J.C. & Matias, D.M. (1995).** Effects of water regime on the distribution of *Meloidogyne graminicola* and other root-parasitic nematodes in a rice field

toposequence and pathogenicity of *M. graminicola* on rice cultivar UPL R15. *Nematology*, **41**: 219-228.

- Ramakrishana, S. & Rajendran, G. (1999).** Changes induced by *Meloidogyne incognita* and *Rotylenchulus reniformis* individually and in combination on physiology, chlorophyll and nutrient content of papaya. *Nematologia Mediterranea*, **27**:119-122.
- Rao, Y.S, Prasad, J.S. & Panwar, M. S. (1986).** Nematode problems in rice: crop losses, symptomatology and management. In: Swarup G, Dasgupta DR (eds) Plant Parasitic Nematodes of India: Problems and Progress, IARI, New Delhi, India, pp. 179-299.
- Rao, S. Y., Jayaprakash, A. & Mohan, J. (1988).** Nutritional disorders in rice due to infestation *Hirschmanniella oryzae* and *Meloidogyne graminicola*. *Revue de Nematologie*, **11**:375-380.
- Sammut. M. , Noack, Y., Rose, J., Hazemann, J., Proux, O., Ziebel, D. M. & Fiani, E. (2010).** Speciation of Cd and Pb in dust emitted from sinter plant. *Chemosphere*, **78**: 445–450.
- Sanyal, S.K., Jeevan Rao, K. & Sadana, U.S. (2012).** Toxic Elements and Other Pollutants –A Threat to Nutritional Quality; *Proceedings of the Platinum Jubilee Symposium on Soil Science in Meeting the Challenges to Food Security and Environmental Quality*, Indian Society of Soil Science, New Delhi. pp. 226.
- Schoeneweiss, D. F. (1978).** Water stress as a pre-disposing factor in plant disease. In *water deficits and plant growth* 5. (Ed. T. T. Kozlowiski) 61-69. Academic Press, Newyork, San Fransisco, London.
- Schoof, R.A., Yost, L.J., Eickhoff, J., Crecelius, E.A., Meacher, D.M. & Menzel, D.B. (1999).** A Market basket survey of inorganic arsenic in food. *Food Chemistry and Toxicology*, **37**: 839–846.
- Shafiee, M. F. & Jenkins, W.R. (1963).** Host-parasite relationships of *Capsicum frutescens* and *Pratylenchus penetrans*, *Meloidogyne incognita acrita*, and *M. hapla*. *Phytopathology*, **53**: 325-328.
- Shahid, M. , Pinelli, E., Pourrut, B., Silvestre, J. & Dumat, C. (2011).** Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation. *Ecotoxicology and Environment Safty*, **74**: 78–84.

- Sharma, P. & Dubey, R.S. (2005).** Lead toxicity in plants. *Brazilian Journal of Plant Physiology*, 17: 35-52.
- Singh, I., Gaur, H.S., Briar, S.K., Sharma, S.K. & Sakhuja, P.K. (2003).** Role of wheat in sustaining *Meloidogyne graminicola* in rice-wheat cropping system. *International Journal of Nematology*, 13: 79-86.
- Swain, B.N. & Prasad, J.S. (1988).** Chlorophyll content in rice as influenced by the root-knot nematode, *Meloidogyne graminicola* infection. *Current Science*, 57: 895-896.
- Takahashi, Y., Minamikawa, R., Hattori, K.H., Kurishima, K., Kihou, N. & Yuita, K. (2004).** Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. *Environmental Science & Technology*, 38: 1038-44.
- Trudgill, D. L., Evans, K. & Parrott, D. M. (1975).** Effects of potato cyst-nematodes on potato plants: effects on haulm size, concentration of nutrients in haulm tissue and tuber yield of a nematode resistant and a nematode susceptible potato variety. *Nematologica*, 21:183-191.
- Uzu, G., Sobanska, S., Sarret, G., Munoz, M. & Dumat, C. (2010).** Foliar lead uptake by lettuce exposed to atmospheric fallouts. *Environmental Science & Technology*, 44: 1036-1042.
- Vahter, M., Concha, G., Nermell, B., Nilsson, R., Dulout, F. & Natarajan, A.T. (1995).** A unique metabolism of inorganic arsenic in Native Andean women. *European Journal of Pharmacology*, 293: 455-462.
- Vast, P. H., Caswell, E. P. & Zasoskic, R. J. (1998).** Effects of two endoparasitic nematodes (*Pratylenchus coffeae* and *Meloidogyne konaensis*) on ammonium and nitrate uptake by Arabica coffee (*Coffea arabica* L.). *Applied Soil Ecology*, 10: 171-178.
- Verma, K. K. & Jain, R. K. (2006).** Nutrient uptake as affected by inorganic fertilizers under *Meloidogyne incognita* infested conditions in cotton. *Indian Journal of Nematology*, 36:60-64.
- Verma, S. & Dubey, R.S. (2003).** Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Science*, 164: 645- 655.
- Vetterlein, D., Szegedi, K., Ackermann, J., Mattusch, J., Heinz-Ulrich, Hartmut, N., Tanneberg & Jahn, R. (2007).** Competitive Mobilization of Phosphate and

Arsenate Associated with Goethite by Root Activity. *J. Environ. Qual.* **36**:1811–1820.

- Wang, Z. S, Chen, X. P., & Wang, X. J. (2010).** The effect of anaerobic redox cycling of iron on arsenic mobility in paddy. *Asian J Ecotoxicol*, **5**: 862–867.
- Welsh, R.P.H. & Denny, P. (1980).** The uptake of lead and copper by submerged aquatic macrophytes in two English lakes. *Journal of Ecology*, **68**: 443- 455.
- Williams, P.N., Prince, A.H., Raab, A., Hossain, S.A., Feldmann, J. & Meharg, A.A. (2005).** Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. *Environmental Science & Technology*, **39**: 5531–5540.
- Williams, P.N., Villada, A., Deacon, C., Raab, A. & Figuerola, J. (2007).** Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environmental Science & Technology*, **41**: 6854–59.
- Weissenhorn, C., Leyval, G., Belgy & Berthelin, J. (1995).** Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. *Mycorrhiza*, **5**: 245-251.
- Xu, X.Y., McGrath, S.P., Meharg, A. & Zhao, F.J. (2008).** Growing rice aerobically markedly decreases arsenic accumulation. *Environmental Science & Technology*, **42**: 5574–79.
- Yang, J.R., Bao, Z.P. & Zhang, S.Q. (1993).** The distribution and binding of Cd and Pb in plant cell. *China Environmental Science*, **13**: 263-268.
- Yang, M. G., Lin X.Y. and Yang. X.E. (1998).** Impact of Cd on growth and nutrient accumulation of different plant species. *Chinese Journal of Applied Ecology*, **19**: 89-94.
- Zaman, M. S. and Zereen, F. (1998).** Growth responses to radish plants to soil cadmium and lead contamination. *Bulletin of Environmental Contamination and Toxicology*, **61**: 44-50.
- Zhang, W.D., Liu, D.S., Tian, J.C. & He, F.L. (2009).** Toxicity and accumulation of arsenic in wheat (*Triticum aestivum* L.) varieties of China. *International Journal of Experimental Botony*, **78**: 147-154.
- Zhao, F.J., Ma, J.F., Meharg, A.A. & McGrath, S.P. (2009).** Arsenic uptake and metabolism in plants. *New Phytologist*, **181**: 777–794.

Zheng, R.L., Sun, G.X. & Zhu, Y.G. (2013). Effects of microbial processes on the fate of arsenic in paddy soil. *Chinese Science Bulletin*, **58**: 186-193.