

भारतीय कृषि अनुसन्धान संस्थान के सीवेज प्रदूषित भूमि पर आर्द्रभूमि द्वारा  
साफ किये गए सीवेज पानी के अनुप्रयोगों का उसकी मिट्टी के स्वास्थ्य एवं कृषि  
उपज पर प्रभाव

**IMPACT OF WETLAND-TREATED SEWAGE WATER  
APPLICATIONS ON SOIL HEALTH & AGRICULTURAL PRODUCE  
FROM EXPERIMENTAL SEWAGE PLOT SITE OF INDIAN  
AGRICULTURAL RESEARCH INSTITUTE**

By

**PARITOSH KUMAR**



**DIVISION OF ENVIRONMENTAL SCIENCES  
INDIAN AGRICULTURAL RESEARCH INSTITUTE  
NEW DELHI – 110012**

**2012**

**Impact of Wetland-Treated Sewage Water Applications on  
Soil Health & Agricultural Produce from Experimental Sewage Plot Site  
of Indian Agricultural Research Institute**

**By**

**PARITOSH KUMAR**

A Thesis Submitted to the Faculty of Post-Graduate School,  
Indian Agricultural Research Institute, New Delhi  
in partial fulfillment of requirements for the degree of

**MASTER OF SCIENCE  
IN  
ENVIRONMENTAL SCIENCES  
(2012)**

**Approved by:**

**Advisory Committee**

**Chairperson : Dr. (Mrs.) Ravinder Kaur**

---

**Co-chairperson: Dr. S D Singh**

---

**Members: Dr. Chandrasekharan Hariharan**

---

Dr. S.K. Yadav

---



**Division of Environmental Sciences  
Indian Agricultural Research Institute,  
New Delhi-110012**



**Dr. Ravinder Kaur**

*ICAR National Fellow, Division of Environmental Sciences &  
Project Director, Water Technology Centre,  
Indian Agricultural Research Institute, New Delhi -110012*

**CERTIFICATE**

This is to certify that the thesis entitled “**Impact of Wetland-Treated Sewage Water Applications on Soil Health and Agricultural Produce from Experimental Sewage Plot Site of Indian Agricultural Research Institute**” submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi in partial fulfilment of **MASTER OF SCIENCE in ENVIRONMENTAL SCIENCES**, embodies the results of *bona fide* research work carried out by **Mr. PARITOSH KUMAR, Roll No. 20059**, under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

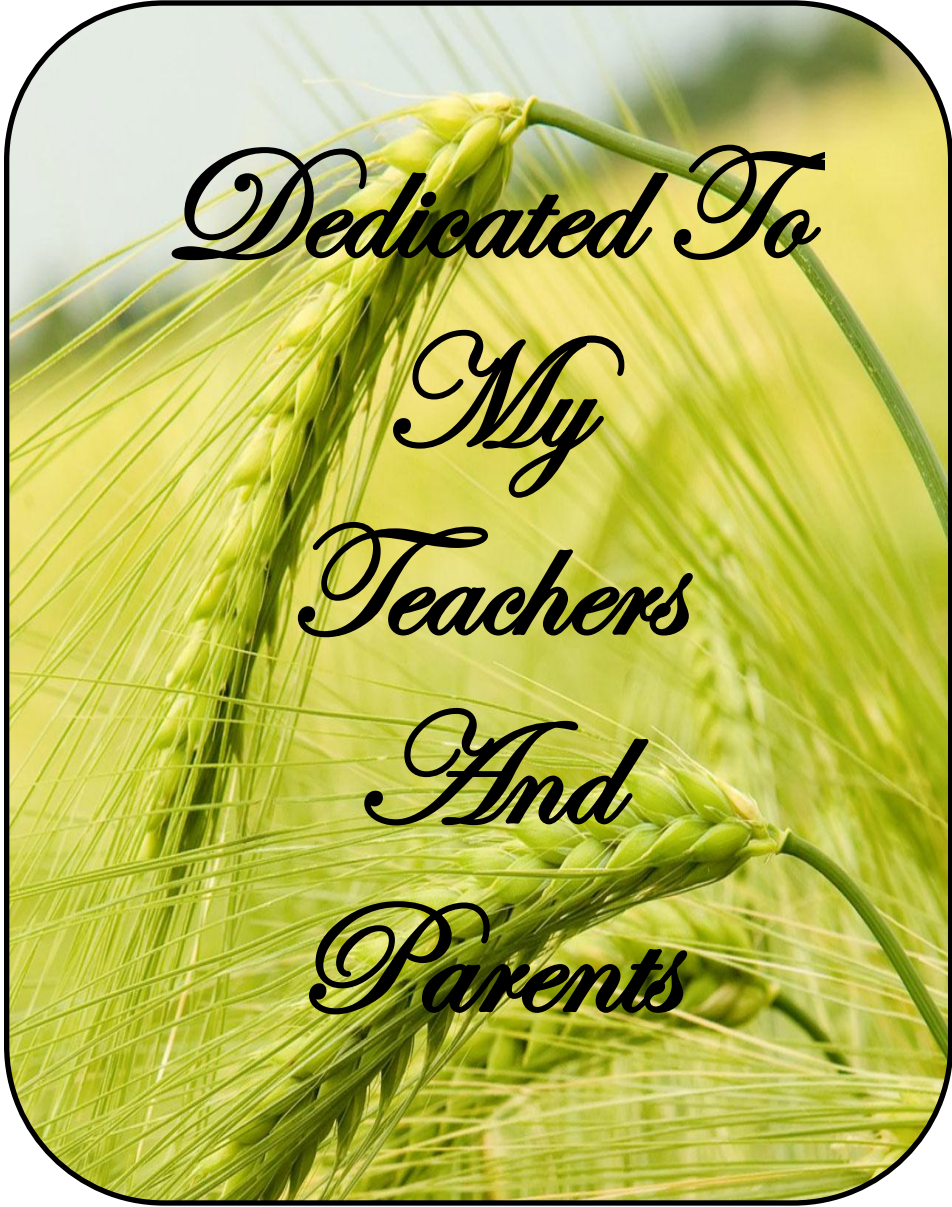
---

**Dr. Ravinder Kaur**

Chairperson (Advisory committee)

**Date:** 30<sup>th</sup> June, 2012

**Place:** New Delhi, India



*Dedicated To*

*My*

*Teachers*

*And*

*Parents*

## ACKNOWLEDGEMENT

---

*I would like to express my deepest sense of gratitude and indebtedness to my chairperson, Dr. Ravinder Kaur, Project Director (Water Technology Centre) and ICAR National Fellow, Division of Environmental Sciences, Indian Agricultural Research Institute, New Delhi for her invaluable guidance, regular encouragement, cooperative attitude, immense patience, useful discussions and peerless criticisms during the entire course of investigation and preparation of this document and for being a consistent source of inspiration towards the completion of this work.*

*It is my privilege to express profound sense of gratitude to my all advisory committee members as well viz. Dr. S.D. Singh (Professor and Principal Scientist, Division of Environmental Sciences); Dr. S.K. Yadav (Senior Scientist, SST) and Dr. Chandrasekharan Hariharan (Head, USI) for their constructive and valuable suggestions, support and encouragement during the research work.*

*My sincere thanks are also due to Dr. H.C. Joshi (former Head), Dr. R.K. Rattan (Head), entire faculty of Division of Environmental Sciences and to Mrs. Usha Rani, PS to Professor, for her assistance in timely completion of all academic formalities.*

*I would also like to thank Ms. Laishram Gitla, Ms. Deepa, Dr. Parveen Sachdeva, Dr. Jagpal Singh and Sonu, Shaam and Raju for sharing their shoulders during the entire span of research work. I would also like to place on record my thanks to my all seniors, especially to Gaurav Dhir Sir, who was always with me as my shadow and to my friends Fayaz, Nishant, Sheetal and Sweeta for sharing very memorable moments during my entire period of study/research at IARI.*

*My special thanks are also due to my beloved parents for their endless love, affection, sacrifice and constant inspiration that enabled me to pursue my goals and to never admit defeat; to my almighty God for giving me the opportunity and to the institute for providing me the necessary financial assistance in the form of IARI Fellowship.*

Place: New Delhi

Dated: 30<sup>th</sup> June 2012

*Paritosh Kumar*

## CONTENTS

<b>S. No.</b>	<b>Title</b>	<b>Page No.</b>
1.	Introduction	1-9
2.	Background	10-41
3.	Materials and Methods	42-68
4.	Research Paper -1	69-96
5.	Research Paper -2	97-132
6.	Research Paper -3	133-147
7.	Summary and Conclusion	148-150
8.	Abstract	151-152
9.	Appendix	153-158
10.	Bibliography	I-XXI

## LIST OF TABLES

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
3.1	General characteristics of IARI micro-watershed	44
3.2	List of physico-chemical test performed on the water samples	51
3.3	List of physico-chemical test performed on soil samples	53
3.4	List of parameters performed on plant samples	54
4.1	Contamination factor of different metals with respect to ground water ( $CF_{GW}$ ) for paddy	77
4.2	Contamination factor of different metals with respect to sewage water ( $CF_{SW}$ ) for paddy	77
4.3	Contamination factor of different metals with respect to ground water ( $CF_{GW}$ ) for wheat	78
4.4	Contamination factor of different metals with respect to sewage water ( $CF_{SW}$ ) for wheat	78
4.5	Pollution Load Index with respect to sewage water ( $PLI_{SW}$ ) of wheat and paddy	79
4.6	Pollution Load Index with respect to ground water ( $PLI_{GW}$ ) of wheat and paddy	79
5.1	Bio-concentration factor of various metals in wheat	105
5.2	Bio-concentration factor of various metals in paddy	106
5.3	Translocation Factor (TF) of various metals in wheat	107
5.4	Translocation Factor (TF) of various metals in paddy	108

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
5.5	Enrichment factor (EF) of various metals in paddy grain	110
5.6	Enrichment factor (EF) of various metals in wheat grain	110
6.1	Dietary Intake (DI) of various metals through wheat (mg/kg/day)	139
6.2	Dietary Intake of various metals through paddy (mg/kg/day)	139
6.3	Health Risk Index (HRI) by different metals intake through paddy in an adult human	141
6.4	Health Risk Index (HRI) by different metals intake through wheat in an adult human	141
6.5	Hazard Index (HI) of various metals intake through paddy and wheat in an adult human	141

### **APPENDIX**

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
9.1	Recommended irrigation water quality parameters	153-154
9.2	Recommended safe limit of different parameters for soil	155
9.3	Recommended safe limit of different metals for agricultural produce (crop)	156
9.4	Safe limit of different parameters for agricultural produce (vegetables)	157
9.5	Recommended limit of different metals in plant	158
9.6	Oral reference dose of different metals	158

## LIST OF FIGURES

Fig No.	Title	Page No.
3.1	Location of project site within IARI micro-watershed	43
3.2	Experimental field layout	45
3.3	(a) Experimental wastewater treatment system based on vertical sub-surface flow (VSSF) constructed wetland technology (b) Experimental micro-plots	46
4.1	Monthly variation in (a) Temperature, (b) Dissolved Oxygen and (c) Turbidity in different irrigation water	81
4.2	Monthly variation in (a) Fluoride (b) pH and (c) Oxidation Reduction Potential value in different irrigation water	82
4.3	Monthly variation in (a) Total Dissolved Solid, (b) Electrical Conductivity and (c) Salinity in different irrigation water	83
4.4	Monthly variation in (a) Nitrate, (b) Phosphate and (c) Sulphate concentration in different irrigation water	84
4.5	Monthly variation in (a) Carbonate, (b) Bi-carbonate and (c) Chloride concentration in different irrigation water	85
4.6	Monthly variation in (a) Sodium, (b) Potassium and (c) Calcium and Magnesium concentration in different irrigation water	86
4.7	Monthly variation in (a) Sodium Adsorption Ratio and (b) Residual Sodium Carbonate value in different irrigation water	87
4.8	Monthly variation in (a) Mn, (b) Zn and (c) Fe concentration in different irrigation water	88
4.9	Monthly variation in (a) Ni and (b) Pb concentration in different irrigation water	89
4.10	Monthly variation in (a) Cr-total, (b) Cr-VI and (c) Cr-III concentration in different irrigation water	90
4.11	Variation in soil (a) EC <sub>w</sub> , (b) ESP, (c) Potassium, (d) Nitrate and (e) Phosphate concentration	91

<b>Fig No.</b>	<b>Title</b>	<b>Page No.</b>
4.12	Variation in soil bioavailable metal (a) Manganese, (b) Zinc and (c) Iron concentration	92
4.13	Variation in soil bioavailable metal (a) Nickel, (b) Chromium and (c) Lead concentration	93
4.14	Variation in soil total metal (a) Manganese, (b) Zinc and (c) Iron concentration	94
4.15	Variation in soil total metal (a) Nickel, (b) Chromium and (c) Lead and (d) Cobalt concentration	95
4.16	Contamination factor of various metals in different irrigation plots in Paddy (a) & (b) and Wheat season (c) & (d) with respect to ground water ( $CF_{GW}$ ) and sewage water ( $CF_{SW}$ ), respectively.	96
4.17	Pollution load index of different irrigation plots in Wheat (Rabi) and Paddy (Kharif) season (a) Combined Wheat and Paddy $PLI_{SW}$ , (b) Combined Wheat and Paddy $PLI_{GW}$ (c) Comparative $PLI_{SW}$ of Paddy and (d) Comparative $PLI_{SW}$ of Wheat with respect to previous year	97
5.1	(a) Paddy (Biological yield), (b) Wheat (Biological yield), (c) Paddy (Economic yield) and (d) Wheat (Economic yield)	111
5.2	(a) Paddy (Plant parts length), (b) Wheat (Plant parts length), (c) Paddy (Plant parts dried weight) and Wheat (Plant parts dried weight)	112
5.3	(a) Paddy (Grain per panicle), (b) Paddy (panicle length), (c) Paddy (plant tillers), (d) Paddy (100 seed weight) and (e) Paddy (Test Weight)	113
5.4	(a) Paddy (Fungus infected tillers), (b) Wheat (Termite infected tillers), (c) Paddy (Harvest Index) and (d) Wheat (Harvest Index)	114

<b>Fig No.</b>	<b>Title</b>	<b>Page No.</b>
5.5	(a) Paddy (Germinated plantlets length), (b) Wheat (Germinated plantlets length), (c) Paddy (10 Germinated plantlet weight) and (d) Wheat (10 Germinated plantlet weight)	115
5.6	(a) Paddy (Vigour index-I), (b) Paddy (Vigour Index-II) (c) Wheat (Vigour index-I) and (d) Wheat (Vigour Index-II)	116
5.7	Bio-concentration factor of metals (a) Manganese, (b) Zinc and (c) Iron in Wheat grown on different irrigation plots	117
5.8	Bio-concentration factor of metals (a) Nickel, (b) Lead and (c) Chromium in Wheat grown on different irrigation plots	118
5.9	Bio-concentration factor of metals (a) Manganese, (b) Zinc and (c) Iron in Paddy grown on different irrigation plots	119
5.10	Bio-concentration factor of metals (a) Nickel, (b) Lead, (c) Cobalt and (d) Chromium in Paddy grown on different irrigation plots	120
5.11	Translocation factor of metals (a) Manganese, (b) Zinc and (c) Iron in Wheat grown on different irrigation plots	121
5.12	Translocation factor of various metals (a) Nickel, (b) Lead, (c) Cobalt and (d) Chromium in Wheat grown on different irrigation plots	122
5.13	Translocation factor of various metals (a) Manganese, (b) Zinc and (c) Iron in Paddy grown on different irrigation plots	123
5.14	Translocation factor of various metals (a) Nickel, (b) Lead, (c) Cobalt and (d) Chromium in Paddy grown on different irrigation plots	124
5.15	Enrichment factor for (a) Paddy and (b) Wheat	125
5.16	Wheat plant tissue concentration of various metals (a) Mn, (b) Zn and (c) Fe grown on different irrigation plots	126

<b>Fig. No.</b>	<b>Title</b>	<b>Page No.</b>
5.17	Wheat plant tissue concentration of various metals (a) Cr-total, (b) Cr-VI and (c) Cr-III grown on different irrigation plots	127
5.18	Wheat plant tissue concentration of various metal (a) Ni, (b) Pb and (c) Co grown on different irrigation plots	128
5.19	Paddy plant tissue concentration of various metal (a) Mn, (b) Zn and (c) Fe grown on different irrigation plots	129
5.20	Paddy plant tissue concentration of metal (a) Cr-total, (b) Cr-VI and (c) Cr-III grown on different irrigation plots	130
5.21	Paddy plant tissue concentration of various metal (a) Ni, (b) Pb and (c) Co grown on different irrigation plots	131
5.22	Seed germination status of Wheat and Paddy grown on different irrigation plots	132
6.1	Daily intake of various metals (a) Nickel, (b) Lead, (c) Chromium, (d) Manganese, (e) Zinc and (f) Iron through Paddy grains grown on different irrigation plots along with their oral reference dose	142
6.2	Daily intake of various metal (a) Nickel, (b) Lead, (c) Chromium and (d) Cobalt through Wheat grains grown on different irrigation plots along with their oral reference dose	143
6.3	Daily intake of various metals (a) Manganese, (b) Zinc and (c) Iron through Wheat grains grown on different irrigation plots along with their oral	144
6.4	Health risk Index (HRI) of (a) Paddy and (b) Wheat	145
6.5	Hazard Index of (a) Paddy, (b) Wheat and (c) Combined wheat and paddy crop	146
6.6	(a) Hazard Index (Wheat), (b) Hazard Index (Paddy) and Comparative Hazard Index (Wheat- Paddy)	147

## INTRODUCTION

---

Water is an essential substance upon which all life depends. Where there is water there is life and where water is scarce, life has to struggle. Thus water is one of the precious resources on the earth which is required for the existence of any form of life. It is flowing on the planet just like the blood in our body which not only carries nutrients to each living beings but also dilutes and removes the undesired products from the site of generation to the suitable sink. As per different theories of origin of life, the first life on planet earth originated in water. For instance the classic “Miller and Urey” experiment, that was conducted in 1952 at the University of Chicago for testing the chemical origin of life, clearly demonstrates and supports the Alexander Oparin and J.B.S. Haldane's hypothesis that water is one of the important inorganic constituents on the primitive earth that favoured chemical reactions and thus synthesized organic compounds from their inorganic precursors. Historical evidences also speak of water's importance, as all ancient human civilizations evolved near major river banks viz. Mesopotamian civilization (3200 to 2350 B.C.E.) near Tigris and Euphrates rivers; Egyptian civilization (3100 to 2660 B.C.E.) along river Nile; Harappan civilization (3000 to 1500 B.C.E.) in Indus Valley and Chinese civilization (7000 to 5000 B.C.E.) near Yellow and Yangtze river.

According to UNESCO report on “Water Ethics and Water Resource Management” (Liu et al. 2011), about 75% of the earth's surface is covered with water and has an estimated total global water reserve of about 1400 MM Trillion litres. However, about 97% of this reserve is in oceans and is thus salty, while only 3% (about 35 MM Trillion litres) is fresh. Of this fresh water reserve, 68.7% is locked up in glaciers, icecaps and under permanent snow-cover in poles, mountainous regions and in Greenland; 0.8% exists as permafrost i.e. as soil moisture and about 0.1% is in atmosphere. Of the remaining, about 30.1% exists as groundwater and the rest (only) 0.3 % as surface water in river systems, lakes and reservoirs. Groundwater and surface water, which together constitute 30.5% of the freshwater reserves and about 0.76% of the total water on the planet, are the most easily accessible and the most used sources of water. Thus, even though three quarters of the earth's surface is covered with water; less than 1% of it is available with most of it stored underground (99%). Thus, it is estimated that annually only about 9000 to 14000 km<sup>3</sup> are economically available for human use (FAO WATER, 2008).

The daily per capita drinking water requirement works out to be around 2 to 4 litres per person while that required for producing a day's food is around 2000 to 5000 litres per head. This clearly shows that agriculture is by far the largest user of freshwater and accounts for nearly 70% to 90% (in some countries) of all global freshwater extractions (Gleick, 2000; FAO, 2002, 2008). Worldwide the major extractions of water in different sectors constitute Agriculture (70%), Industrial (22%) and Domestic (8%).

The global population is expanding by 80 million people annually (with a rate of 1.2% per annum) and is expected to grow further by two billion by 2030 thereby increasing the demand for freshwater by about 64 billion m<sup>3</sup> (BCM) a year. As a result, the water withdrawals have tripled over last 50 years and the potential global water availability has declined from 12,900 m<sup>3</sup> per capita per year (1970) to 9,000 m<sup>3</sup> (1990) and to 7,000 m<sup>3</sup> (2000). Climate change, especially in developing countries, is also expected to impose an additional stress on water quantity (Magadza, 2000; Kashyap, 2004; Pachauri, 2004). For an increase in temperature of 1°C, agricultural irrigation demand in arid and semi-arid regions of Asia is estimated to increase by at least 10% (Liu, 2002). Thus, water under all use categories is becoming scarce. Presently more than eighty countries, with forty percent of the world's population are facing water shortage.

In a developing urban society, about 80 to 90 percent of the urban water supply to homes, businesses and industries is not consumed by users but is returned to the urban environment as wastewater (Asano et al., 2007). In the developing world, the urban population is expected to increase by 1.4 billion or by 54% and in the lower-income countries the urban population is projected to more than double from 254 million in 2010 to 539 million in 2030. Although reliable data on the extent and severity of pollution is incomplete, one estimate of UN that the amount of wastewater produced annually is about 1,500 km<sup>3</sup>, six times more water than exists in all the rivers of the world (UN WWAP, 2003). Assuming that 1 litre of wastewater pollutes 8 litres of freshwater; the present burden of pollution may be up to 12,000 km<sup>3</sup> worldwide. Our planet has not been facing such large volume of wastewater generated and dispersed today than at any other time in the history. Thus disposal of increasing large volume of sewage wastewater is also a problem of increasing importance throughout the world.

The existing wastewater treatment facilities are not matching with the increasing volume of wastewater. An estimate says, currently more than 3300 water reclamation facilities are working worldwide with varying degrees of treatment and for various applications: agricultural irrigation, urban landscaping and recreational uses, industrial cooling and processing, and indirect potable water production such as groundwater recharge (Aquarec, 2006, FAO, 2010). But most of them are working in the developed countries like Japan (over 1800), USA (over 800) and Australia (450) respectively. Beside this, the high number of dysfunctional treatment plants and low general levels of wastewater treatment in developing countries e.g. about 35% in Asia and 14% in South America creating extra pressure on suitable treatment of wastewater (WHO and UNICEF, 2000). As a result, increasing scarcity of freshwater resources is driving many countries in the arid and semi-arid regions to use marginal quality water for agriculture (Frolov et al., 2004) and related activities. Where wastewater treatment services are not provided, the use of wastewater in agriculture actually acts as a low-cost treatment method, taking advantage of soil's capacity to naturally remove contamination. Furthermore, wastewater reuse may reduce fertilizer rates and provide a low cost source of irrigation water. Both the need to conserve water and to safe and economically dispose of wastewater make the use of wastewater in agriculture a very feasible option besides preventing the pollution of streams and lakes, because it closes the loop between waste disposal and water supply. In India, the use of treated sewage in irrigation was also emphasised in the Water (Prevention and Control of Pollution) Act 1974; however, the State Governments have failed to recognize its importance during the last 37 years.

Worldwide, an estimated 20 million hectares are irrigated with raw or partially diluted wastewater (Jiménez and Asano, 2008; Scott et al., 2004) which is approximately 10% of total irrigated land (FAO Wastewater Database, 2009). However recent FAO report (2010), it is estimated that about 5, 25,000 ha land is irrigated with reclaimed water. In terms of volume, the use of reclaimed water for agricultural irrigation has been reported to be over 15 Mm<sup>3</sup>/d, in at least 44 countries (Jiménez and Asano, 2008). Worldwide more than 800 million farmers are engaged in urban agriculture, of which about 200 million practice markets oriented farming on open spaces and are often using poor-quality irrigation water when good-quality water is not available. The 20 countries with largest volume of waste water use in

agriculture (in m<sup>3</sup>/d) are Mexico (4493000), Egypt (1918000), China (1239000), Syria (1182000), Spain (932000), USA (911000), Israel (767000), Italy (741000), Saudi Arabia (595000), Kuwait (432000), Iran (422000), Chile (380000), Jordan (225000), UAE (200000), Turkey (137000), Argentina (130000), Tunisia (118000), Libya (110000), Qatar (80000) and Cyprus (68000), (Jimenez and Asano, 2008). Reclaimed wastewater use in California for crop or landscape irrigation (California State Water Resources Control Board, 2003) amounts to about 67%; approximately 75% in Israel (Arlosoroff, 2002); 41 % in Japan and 15% in Tunisia.

In terms of area, India occupies third position in the use of untreated wastewater for irrigation; after China and Mexico (Scott et al. 2010). However, at present reliable data on the total land area irrigated with wastewater does not exist. Strauss and Blumenthal (1990) estimated over 73,000 ha in India are irrigated with wastewater. Most wastewater irrigation in India occurs along rivers, which flow through such rapidly growing cities as Delhi, Kolkata, Coimbatore, Hyderabad, Indore, Kanpur, Patna, Vadodara, Varanasi, Dharward, etc. The area along just one river Musi, Hyderabad alone covers approximately 40,500 ha, irrigated with wastewater (Van der Hoek, 2004). So presently the figure on the total area should be much higher than 73,000 ha. In many parts of the world, treated municipal wastewater has been successfully used for the irrigation of various crops including agronomic (Bielorai et al., 1984; Feigin et al., 1984) and horticultural (Neilsen et al., 1989a, b, c and 1991) crops.

At present, at least one-tenth of the world's population consumes crops irrigated with untreated or poorly treated wastewater, mostly in developing countries in Africa, Asia and Latin America (Smit & Nasr, 1992) as it fulfils the need of irrigation water to resource poor farmers. The average cost of sewage supply has been estimated to be around Rs. 188/ha/annum (ICID International Workshop on Wastewater Reuse Management). As compared to the freshwater irrigations, wastewater use has often been reported to produce higher yields therefore it is potentially very profitable for farmers. In the Guanajuato river basin in Mexico, 140 ha of land are irrigated with wastewater, which provides local farmers with nutrients estimated to be worth US\$ 135 per hectare per year (Future Harvest, 2001). Further, in Nagpur (India) irrigation with waste stabilization pond effluents has been reported to yield 28, 8, 47, 30 and 42 % more wheat, moong beans, rice, potato and cotton,

respectively, than irrigation with freshwater supplemented with fertilizer (Shende et al., 1985). As a result, farmers in some areas of Pakistan willingly pay US\$ 350-940 per year for access to wastewater compared with US\$ 170 per year to freshwater (Ensink, Simmons & van der Hoek, 2004). In Hyderabad and Jaipur (India) too farmers purchase sewage water at the rate of Rs. 75 and Rs. 400/ha/year, respectively. However its social acceptability due to bad smell, presence of fecal matter, engine oil, harmful chemicals and some diseases infections after use like blistering, skin infection, injury to hands and lower legs and mosquito nuisance is still low.

Land application of domestic waste water is gaining momentum owing to the fact that land application provides primary, secondary, and tertiary treatment (Idelovitch and Michael, 1984; Smith and Schroeder, 1985). But, wastewater in soil has different fate than in water bodies. Soil has a limited capacity to bio-filter. It cause some affect both in positive & negative ways. The extent of impact depends on the composition of effluent (depends on the origin & season), quantity used, period of application, irrigation type, irrigation frequency, way of use (planned or unplanned use, direct or indirect use, raw or diluted) along with soil type & climatic condition. The practice of uncontrolled use of undiluted wastewater as it provides nutrients or is more reliable or cheaper than other water sources (Scott et al., 2004) can pose a threat to sustainable agriculture, human health and the environment if used for irrigation without taking certain precautions (Qadir et al., 2007) mainly due to not only the associated pathogens, but also heavy metals and other undesirable constituents depending on the source. Farmers, consumers, and some government agencies in many countries are not fully aware of the potential impacts of irrigation with wastewater.

Wastewater use in agriculture builds soil organic carbon (SOC) enriches humic content thereby increasing soil moisture, retaining metals and enhancing microbial activity. Under aerobic conditions, rate of decomposition of organic matter in soils is faster and more complete. Thus, wastewater application under controlled conditions allows biodegradation of hundreds of kilograms of Bio-Chemical Oxygen Demand (BOD) per hectare per day, with no impact on environment (Bouwer & Chaney, 1974). However, when BOD concentrations are extremely high (BOD >500 ppm), combined with high total dissolved solids (TDS) levels, it may leads to soil clogging.

Sodium and other forms of salts are also more persistent in recycled waters and may increase soil salinity due to their long-term land application particularly in arid and semi-arid regions. As sodium concentrations increase, the electrophoretic mobility of the clay platelets increases resulting in swelling dispersion of the clay particles thus impacting on soil permeability (Halliwell et al., 2001). Along with sodium, presence of suspended solids (Magesan et al., 2000), nutrients which cause excess growth of microorganisms in the soil (Magesan et al., 1999) or interaction of dissolved organic matter with the soil profile (Tarchitzky et al., 1999) causes reduction of hydraulic conductivity of soil. This can impact on the ability of water to infiltrate into the soil profile and thus, reduce the water availability to irrigated crops. The salinity of recycled water can thus impact both the soil and the growth of the crops being irrigated. Concentration of carbonates and bicarbonates above 500 mg/l can also cause soil deflocculation. Grain crops such as wheat have been observed to be more resistant to saline irrigation of soils with less decrease in yields over a wide range of electrical conductivities compared with other more sensitive crops (Katerji et al., 2003).

Wastewater contains different types of heavy metals like lead, cadmium, mercury, nickel etc. which are carcinogenic in nature. Some of the harmful impacts of intake of toxic metals become apparent only after several years of exposure (Bahemuka and Mubofu, 1999; Ikeda et al., 2000). Some studies are reported that the consumption of heavy metal contaminated food can deplete some essential nutrients in the body that are further responsible for decreasing immunological defences, intrauterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Iyengar and Nair, 2000; Tu'rkdogan et al., 2003).

Among the numerous hazardous materials heavy metals are extremely persistent in the environment, and they are non-biodegradable and non-thermo-degradable (Cao and Hu, 2000). Long-term use of wastewaters on agricultural lands often results in the build-up of the elevated levels of heavy metals in arable layer of soils. Many studies showed that heavy metals in high concentration have a clear negative impact also on the soil microbial biomass, microbial structure, microbial diversity, bacterial abundance, various enzyme activities and C and N mineralization,

which can pose serious threats to the stabilization and health of soil ecosystems in the long term.

Contamination of soils and crops due to wastewater irrigation are widely reported from different parts of the world e.g. a report says that 45% of wastewater irrigated areas in China are contaminated with heavy metals at the most serious level. Not only in China, this problem emerging in several other countries like Germany, France and India (Ingwersen and Streck, 2006; Dere et al., 2006; Singh and Kumar, 2006). In several studies, it is established that solubility of metals in soils mainly depends on soil pH and organic carbon (Jopony and Young, 1994; Hough et al., 2003; Tye et al., 2003). Generally a metal will not be absorbed by the plants unless it first reaches a threshold concentration in the soil. Metals are bound to soil with pH above 6.5 and high organic matter content. At pH level below this organic matter is consumed and metals become mobile and can be absorbed by crops and contaminate water bodies (WHO, 2006). Extent of build-up of metals in waste water-irrigated soils also depends on the period of its application (Bansal et al., 1992; Palaniswami and Sree Ramulu, 1994). When the capacity of the soil to retain heavy metals is reduced due to repeated use of domestic wastewater, soil can release heavy metals into ground water or soil solution available for plant uptake (Sharma *et al.*, 2007). Cadmium, copper, molybdenum, nickel and zinc are frequently present in wastewater and can be mobilized easily and absorbed by plants.

When plants are exposed to excess heavy metals in soil, they exhibit symptoms of phytotoxicity such as inhibition of seed germination, decreased plant height, decreased tillering, reduced root growth, decreased shoot growth, leaf chlorosis and necrosis, decreased chlorophyll metabolism, and lower fruit and grain yield. Such exposure may also sometimes lead to plant death, as almost all of the above-mentioned adverse effects of heavy metals on physiological and agronomical parameters are related to the basic photochemical reactions in plants (Rahman *et al.*, 2007).

Environmental and human health risks due to wastewater agriculture/ use can be minimized by suitable treatment of wastewater before use, careful handling, wise application and proper awareness. However the available technologies, due to their high capital and maintenance costs, are unaffordable by the developing world. The

processes involved in several conventional treatment systems, except stabilization ponds, are difficult and costly to operate in developing countries due to their requirements for high energy, skilled labour and installation/ operation and maintenance costs (Carr and Strauss, 2001). Thus the developing world is in the need of such non-conventional low cost and environmentally sustainable treatment technologies that have lower capital, maintenance and operational costs and could be operated by less skilled persons.

Non-conventional wastewater treatment methods include the use of low-cost systems such as on-farm ponds, sedimentation traps, bio sand-filters and constructed wetlands. Constructed wetlands are complex, integrated systems in which water, plants, animals, micro-organisms and the environment interact to improve water quality i.e. driven by natural energies of sun, wind, soil, microorganisms, plants and animals (Kadlec and Knight, 1996). These are the beds of aquatic macrophytes that grow in soil, sand or gravel and mainly of three types: surface-flow, horizontal-flow subsurface and vertical-flow systems. The objective of constructing wetlands is to duplicate the processes occurring in natural wetlands. Constructed wetlands mimic nature by mechanically filtering, chemically transforming & biologically consuming potential pollutants in the wastewater stream. It is able to give secondary to tertiary level of treated water with minimum cost and energy. A study conducted by Luederitz et al. (2001) confirmed that constructed wetland is one such sustainable alternative to treat sewage waters. The results of their study indicated that a semi-centralized constructed wetland system needs 83% lesser energy than the central mechanical-biological wastewater system and 72% lesser energy than the large-scale sewage treatment plant. The constructed wetland system required 76% lesser material input than a central mechanical-biological wastewater system and 63% lesser material than a large-scale sewage treatment plant. A similar study was also conducted by Chen et al. (2008) to compare the cost and efficiency of a vertical subsurface-flow constructed wetland system with a conventional sewage water treatment system. Their study confirmed that the cost of wastewater treatment through a vertical subsurface-flow constructed wetland is much lower than that through a conventional treatment plant.

Wetland systems have shown promise in achieving large reductions in nutrient/ toxic levels and in coliform bacteria. In fact, constructed wetlands have often been reported to produce a standard of treatment equivalent to tertiary or advanced

wastewater treatment that is far better than a typical “package plant” or municipal sewage plant that produces effluent at secondary sewage standards quality (Reed and Brown, 1995). However, scanning of literature reveals that:

- Most of the studies on the wetland treated waste waters have been focussed on either evaluation of plant treatment efficiency / capacity or on the cost benefit analysis,
- Most of the existing studies lack comparative comprehensive risk of untreated and treated sewage water use on the soil, crop and consumer health,
- Though cereals have a higher contribution in the diet, yet most risk or impact assessment studies have been focussed on the vegetable crops.

Thus, there are very few studies that can be used for accounting an overall impact of the wetland treated wastewaters on the quality of soil/ agricultural produce and hence risk to human health.

One such wetland technology based pilot sewage water treatment plant (of 1500 LPD capacity) is in operation since November 2009, at the sewage plot site of Indian Agricultural Research Institute (IARI) farmlands. Thus, the main **objectives** of the present study were to:

1. Assess impact of the aforementioned IARI wetland - treated sewage water applications on the soil of the experimental sewage plot site.
2. Assess impact of wetland treated sewage water applications on the agricultural produce from sewage plot site and
3. Assess comprehensive risk of untreated and treated sewage water use on the consumer health.

## BACKGROUND

---

### **Objective: (1) To assess impact of IARI wetland treated sewage water applications on the soil of the experimental sewage plot site**

Wastewater re-use in agriculture has both positive and negative impacts on soil. On one hand the nutrient content (N, P, K and micronutrients) and organic matter increases the soil fertility while on the other hand, excess nutrients, high EC, TDS, TSS, sodium, heavy metals and pathogenic microbes degrade the soil physical, chemical, hydrological and biological properties. The extent of impact depends on the composition of the effluent as influenced by its origin, extent of treatment (preliminary, primary, secondary or tertiary), quantity of use, period of application, irrigation frequency, method of irrigation (flooding or ridge-furrow, sprinkler or drip, surface or subsurface), soil type, climatic condition and usage (i.e. planned or unplanned, direct or indirect, raw or diluted). Impact on soil also depends on its texture and clay/ mineral and organic matter content. Keeping all these points in view, review of global literature revealed the following facts:

Walker and Lin (2008) investigated the long term morphological and functional changes in the soils of the cropped and the forested lands of the Pennsylvania State University (USA) that were subjected to wastewater use (about 50 mm/week by spray irrigation) since 1962. The results illustrated an increase in both organic matter content and soil pH by >1 % and by 1-2 units, respectively since 1971. A continuous soils saturation and soil transport, was reflected by the distribution of redoximorphic features and A-horizon thickness across the study area. The depth of the A-horizon was significantly greater in the depressions, while the midslope position had the highest manganese oxide coating percentage, and the summit position had the highest bulk density. The depression areas had the highest mean surface Ksat (10.2 cm/h), while the summit areas had the lowest mean surface Ksat (1.2 cm/h). Overall, although soil properties have changed, the wastewater irrigation system remains functional in this area and the soils are still performing reasonably well.

Bhardwaj et al. (2007) conducted a study in Kibutz Mizra, Israel where an experimental citrus orchard has been irrigated with freshwater (FW) and treated wastewater (TWW) for the last 23 years with 7600 m<sup>3</sup>/ha/yr. With two types of irrigation systems drip irrigation and micro-sprinklers with the objectives to determine the effects of long term irrigation with TWW vs. (FW), and drip vs. microsprinkler

method of irrigation on the saturated hydraulic conductivity (HC) of intact and repacked soil samples, and their relationship with aggregate stability. Initial HC values were found in the range of 0.025–0.25 mm/h for the intact cores and 1.5–16 mm/h in the repacked soil columns. In both types of measurement steady state HC was significantly lower than the initial HC. The value of both Initial HC & steady state HC was lower in case of TWW over FW irrigated soil. Water quality had a greater impact when intact cores were used, while method of irrigation had a greater impact in the case of repacked soil columns. Irrigation water quality and method of irrigation did not have conclusive effects on aggregate stability.

Coppola, et al. (2004) conducted a study in South Sardinia to provide experimental evidence of the consequences of urban wastewater reuse in irrigation practices on the hydrological behaviour of soils. A disturbed layer at the soil surface, which expands in depth with time, was observed, characterized by reduced soil porosity, translation of pore size distribution towards narrower pores and consequent decrease in water retention, hydraulic conductivity and hydrodynamic dispersion. Resulting, the soil beneath the disturbed layer, a unsaturated conditions developed means higher residence times of solutes which may affect the solute transport in soils, as, even of those normally characterized by considerable mobility (e.g. boron), which may accumulate along the profile.

Mandal et al. (2008) conducted a study in Bet She'an Valley, Israel to compare the impact on soil hydraulic properties by 3 years of irrigation with treated wastewater (TWW), the saline–sodic Jordan River water (JRW), moderately saline–sodic spring water (SPW) and a non-cultivated area (control). The HC values for the TWW treatment were found similar to, or lower than the control and significantly higher than those for the JRW treatment. The runoff and soil loss from the TWW treatment were comparable with the control and significantly lower than those from the JRW treatment. TWW could have favourable effects on soil structural stability over Saline–sodic irrigation water, especially in regions with low to moderate rain intensities and/or use of low intensity irrigation systems.

Jalali et al. (2008) were conducted a soil column study with two soil to assess the effects of irrigation with wastewater on soil and groundwater quality in Iran. The average exchangeable sodium percentage (ESP) of the soils was found increased during leaching from 9 to 21 and 28.8 to 29.7 after applying 5.0 and 3.5 L (about 7

and 6 pore volumes) of wastewater to the soils columns, respectively. Salinity of the soils was also increased with the application of wastewater. When the soil columns were leached with distilled water the flow rate of one soil was decreased to zero after 2.2 pore volume indicating damage to soil structure. The final leached concentrations showed that irrigation with wastewater increases the rate of soil sodification of shallow groundwater.

Coronado et al. (2011) conducted a study in Biar (Alicante, SE Spain), with three treatments: fresh water (control), and treated wastewaters from secondary and tertiary treatment to evaluate the short-term effects on grape grown soil for 2 years. They found a slight increase in soil organic carbon content, electrical conductivity (EC) and available Na content in plots irrigated with the secondary treatment. Laboratory analysis showed the decrease in aggregate stability for all treatments due to the wetting and drying cycles associated with each irrigation dose. Changes in microbial biomass carbon and basal soil respiration were also found.

Heidarpour et al. (2007) investigated the effects of wastewater on soil chemical properties using two irrigation methods (subsurface irrigation with porous pipe and surface irrigation) in 2005 at Isfahan, central Iran. The soil EC, Na and Mg of the first layer of soil (0–15 cm) were found significantly greater with subsurface irrigation than with surface irrigation. The EC, Ca and Mg of second and third soil layers irrigated with wastewater were less as compared with groundwater. The amount of K in the first and second soil layers irrigated with wastewater was significantly greater than those irrigated with groundwater. There was no significant effect on soil Na, P and TN due to irrigation with wastewater.

Rattan et al. (2005) conducted a case study to assess the long-term effect of (untreated) sewage irrigations on the heavy metal content in the soils, plants and groundwater in the peri-urban agricultural lands under Keshopur Effluent Irrigation Scheme (KEIS) of Delhi, India. The soil pH was found dropped by 0.4 unit while the organic carbon content of soil increased from 38 to 79% in sewage-irrigated soils as compared to tubewell water-irrigated soil. Sewage irrigation for 20 years resulted into significant build-up of DTPA extractable Zn (208%), Cu (170%), Fe (170%), Ni (63%) and Pb (29%) in sewage-irrigated soils over adjacent tubewell water irrigated soils, whereas Mn was depleted by 31%. Soils receiving sewage irrigation for 10 years exhibited significant increase in Zn, Fe, Ni and Pb, while only Fe in soils was positively affected by sewage irrigation for 5 years. Among these metals, only Zn in

some samples exceeded the phytotoxicity limit. Even after higher build-up of heavy metals in bioavailable pools of sewage-irrigated soils had not found any threat by long term use of sewage effluent but it needs to be monitored periodically to prevent significant build-up.

Al Omron et al. (2012) also got same type of results in the soils of the date palm at Al-Hassa Governorate, Saudi Arabia after 13 years of irrigation. The increase in organic matter content was found from 17% to 30% and the soil pH dropped by 0.3 as a result of sewage irrigation and significant build-up of total concentration of Zn (130%), Pb (55%), Fe (82%), Ni (84%), Mn (30%), Cu (40%), Cr (75%), Co (78%) and As (67%) in sewage-irrigated soil samples over adjacent well water-irrigated soil samples.

Hussain et al. (2010) conducted a survey in Faisalabad, Pakistan where raw city effluent was used for irrigation from more than 25 years to appraise Cd concentration in these waters, soil and its uptake by cereal and legume crops as compared with the tube well and canal water irrigated areas. About 11.0 and 3.7 times higher Cd was found in wastewater and 248% and 260% higher Cd contents at 0-15 cm depth of soils compared to tube well and canal waters irrigated soils. The highest wastewater Cd concentration was found 1.4 µg/L at Malkhanwala and maximum AB-DTPA extractable Cd (0.30 mg/kg & 0.248 mg/kg) was found in soil samples was collected from 0-15 cm depths at Uchkera and Ghulam Muhammad Abad, respectively. In all the cases, Cd was within safe limits and about 70% of the metal was found deposited in upper 30 cm layers.

Saha et al. (2010) conducted a field experiment to evaluate the impact of domestic sewage water on Vertisol with wheat-soyabean cropping system at Bhopal, India. About 150 cm of sewage irrigation over a period of 5 years resulted in significant increases in salinity as well as available fractions of N, P, K, and micronutrients, viz., Zn, Fe, and Mn in soils. Carbon and phosphorus were accumulated more in subsoil layer compared to topmost plough layer. Soil microbiological activity, as indicated by soil respiration, microbial biomass C, as well as dehydrogenase enzyme activity was found higher in sewage water-irrigated soils. There was also significant increase in fungal and actinomycetes as well as total coliform population in the soils. Overall by municipal sewage water irrigation did not found any appreciable harmful effect on soil quality as well as crop productivity;

rather, it proved beneficial in improving soil fertility, wheat productivity, and produce quality.

Singh et al. (2012) conducted an experiment for a year in Nagpur, India by growing wheat (AKW-1071), Gram (Jacky-9218), Palak (Pusa Jyoti), Methi (Kasuri) and Berseem (Multicut) with groundwater and domestic wastewater irrigation. By the use of the domestic waste water it was observed the improvement in the physicochemical properties of the soil and not found a significant effect apart from, slight changes in salt solubility and alkalinity on a clay soil.

Chung et al. (2011) conducted a pot experiment in a plastic film house in Korea to evaluate the translocation and uptake of heavy metals (Pb, Cd, Cu, and Zn) into brown rice (*Oryza sativa* L.) and the heavy metals residues in soils by irrigated with domestic wastewater for a long time (3 years). It was found a slight increase in the level of Pb, Cd, Cu, and Zn with the range  $5.10 \pm 0.01$ ,  $0.105 \pm 0.017$ ,  $5.76 \pm 0.42$ , and  $23.56 \pm 1.40$  mg/kg, respectively in the domestic wastewater-irrigated soil. However, the continuous monitoring and pollution control of hazardous materials from domestic wastewater are needed in order to prevent excessive build-up of heavy metals in the food chain.

Yan et al. (2008) conducted a study in heavy metals polluted (mainly Cd & some Zn & Cu) Zhangshi wastewater irrigation area in Shenyang (China), an area with a 30-year wastewater irrigation history to evaluate the status of heavy metal pollution and the soil microbial characteristics under long-term heavy metal stress. Even though wastewater irrigation ceased in 1993, due to heavy metal stress, soil microbial biomass and soil metabolic quotient ( $qM$ ) decreased, soil metabolic quotient ( $qCO_2$ ) increased, while soil substrate-induced respiration (SIR), dehydrogenase activity (DHA), cellulase activity, and culturable microbial populations did not show persistent responses to the pollution level. The effects of soil heavy metals were more obvious in spring and summer than in autumn. Soil nutrients, except for phosphorous, showed positive effects on soil microbial characteristics, which to a certain degree obscured the adverse effects of soil heavy metals. Soil Cd contributed more to the soil microbial characteristics, but  $qM$  and  $qCO_2$  were more sensitive and showed persistent responses to heavy metals stress.

Zhang et al. (2008) conducted a study in Shandong (China), on an area which have more than 30 years of sewage irrigation history to evaluate the effect of long term sewage irrigation on the soil microbial structural & functional characters with

the methods of Biolog and FAME. It was found that the microbial utilization of carbon sources in sewage irrigation areas was much higher than that of ground water irrigation area (control area). Due to sewage irrigation, microbial functional diversity was found slightly increased by the Biolog analysis; however, the amount of epiphyte decreased by the FAME analysis. The Cr & Zn contents were showed positively correlated with the values of Average Well Coloured Development (AWCD) and the microbial diversity, while Hg content showed negative correlation with the AWCD and Shannon index. Thus heavy metals in the soil irrigated with sewage had not reached to the hazardous concentration and gave positive effects on the microbial community in the soil and improved the soil quality (Soil organic matter, CEC, total nitrogen and total phosphorus significantly increased) but, a regular monitoring is necessary because it the changes of soil microorganisms and soil quality with long term sewage irrigation.

Truu et al. (2009) conducted a study in Kambja (Estonia) to access the effect of secondary-treated wastewater irrigation on willow coppice soil microbial parameters. After 2 years, a significant increase had occurred in soil microbial biomass, respiration and nitrogen mineralization activity (2 times), alkaline phosphatase activity, soil potassium conc. & organic matter content and in upper 10 cm soil while not significant increase observed in potential nitrification, acid phosphatase activity and microbial community metabolic activity.

From the co-effect of municipal wastewater and willow plants on soil microbial community is positive & promote the wastewater purification processes.

Yadav et al. (2002) conducted a study in Kurukshetra, Haryana (India) to access the impact of about three decades of irrigation with domestic sewage effluent on alluvial soil & their effects on the composition of crops and ground waters. They were found build up in total N was up to 2908 kg/ha, available P (58 kg/ha), total P (2115 kg/ha), available K (305 kg/ha) and total K (4712 kg/ha) in surface 0.15 m soil and also organic matter (1.24–1.78%). Traces of NO<sub>3</sub>-N (up to 2.8 mg/l), Pb (up to 0.35 mg/l) and Mn (up to 0.23 mg/l) could also be observed in well waters near the disposal point which indicating the initiation of ground water contamination. Though Long-term irrigation with sewage water adds large amounts of carbon, major and micro- nutrients to the soil but there is a need for continuous monitoring of the concentrations of potentially toxic elements in soil, plants and ground water.

Gupta et al. (2009) found 83.3% of raw wastewater, 68.2% of treated wastewater, 68.6% of soil of the collected soil samples was contaminated with intestinal helminth ova in the wastewater-irrigated area of Titagarh, Kolkata (India) which contaminates the growing vegetables like 44.2% of vegetables in the study area were found to be positive for helminth ova.

Mandal et al. (2008a) conducted an experiment in a cotton field of the Bet She'an region, Israel, to assess the impact of irrigation water quality on soil quality. After irrigating the field with Jordan river-water, Spring-water, Treated wastewater, and Salty Spring-water, applied via drip irrigation to a calcareous silty clay soil for 3 years. Soil quality indices (SQI) were calculated using a Principal component analysis (PCA) weighting factor and each minimum data set (MDS) indicator scored using linear transformation. The SQI was found for uncultivated soil (2.945), Treated Wastewater (1.471), Spring Water (0.923), Salty Spring water (0.823) and Jordan River water (0.582) irrigated soils and Treated Wastewater were considered as the best, irrigation treatment for soil quality.

Singer & Eshel (2003) conducted a field study to determine the distribution and amount of soil inorganic carbon was changed by the application of treated effluent for irrigation, compared to fresh-water irrigated soils in Bakersfield, California, that had received varying amounts of treated effluent for 70 years. Total carbonate content and clay-size carbonate was found more abundant at sites irrigated with treated effluent (1172 Mg/ha at 2-4 m depth) than at sites irrigated with fresh water (8.7 Mg/ha at 2-4 m depth), indicating that effluent had increased carbonate precipitation. The analysis of isotopes carbon and oxygen indicated that these carbonates were pedogenic in nature &  $^{14}\text{C}$  dating of the carbonates indicated that at the shallow depth, the carbonates were younger compared to deeper in the soil profile. Thus increase soil inorganic carbon sequestration in arid and semiarid region soils and act as another method for balancing the increase in  $\text{CO}_2$  in the atmosphere from anthropogenic activity.

Jouzdan et al. (2007) conducted a lysimeter experiment cultivated with crops & vegetables with treated urban effluent in Nashabia, Syria. A moderate increase of heavy metals (As, Cd, Cr and Pb) mainly in upper 0-45 cm soil depth was observed.

Kalavrouziotis et al. (2008) conducted a greenhouse experiment in Agrinion, Greece, to assess the effect of treated municipal wastewater (TMWW), compared to

the ordinary irrigation water in Broccoli and Brussels sprouts plants, as well as on the physical and chemical properties of the clay loam soil. They got significant increase in some macro- and microelements in the Broccoli soil P (18.36–41.16 mg/kg), Zn (3.61–4.64 mg/kg), and Cd (0.065–1.20 mg/kg), while in Brussels sprouts soil P (20.6–36.32 mg/kg), Zn (2.87–4.83 mg/kg) and Cd (0.06–1.45 mg/kg) but the concentrations of most of them were generally within the accepted critical levels, except for P and Zn and Cd.

Yoon & Kwun (2001) conducted a study in an experimental field at Konkuk University in Seoul, Korea, to examine the effect of reed grown constructed wetland treated sewage irrigation on paddy and its soil. The pH of initial slight acidic soil was increased & reached close to neutral. The electrical conductivity after the first year experiment decreased compared to the initial condition, but increased after the second year. This implies that continuous irrigation of treated sewage could cause salt accumulation in the soil. The cation exchange capacity, soil organic matter content, total nitrogen and total phosphorus concentrations was fairly constant and available phosphorus decreased. The paddy soil was not generally affected, although there was an indication of salt accumulation in the field irrigated with treated sewage.

Feizi (2001) conducted a study in Isfahan, Iran where treated wastewater was used for irrigation for growing cereals & vegetables from about 8 years. The soil concentration of Zn, Mn, Cu and Fe in soil was found higher in fields irrigated with effluent, with Mn and Zn more significant.

Surdyk et al. (2010) irrigated their potato farm with wastewater treated using a conventional sand filter treatment and a sand filter combined with a specific filter for heavy metal removal for three years in Belgrade, Serbia. The soil contents in inorganic elements at the end of the three irrigation years were got similar to the initial state. They concluded that appropriately treated low quality waters with high heavy metal contents can be used for irrigation over several years without significant degradation of soil and produces.

Hulugalle et al. (2004) conducted a study by irrigating wheat-cotton field with treated sewage effluent Narrabri, Australia. The Irrigation with treated sewage effluent caused large increases in nitrate-N, small increases in exchangeable Mg, Na and K, and small decreases in SOC. Salinity increased only in the 0.1-0.6 m depth. Application of 2.5 t/ha of gypsum were also added resulting lower nitrate-N accumulation but did not affect any other soil property or deep drainage.

Parvan and Danesh (2009) were investigated the long-term effects of irrigation with treated wastewater on some chemical soil properties by comparing the two fields one irrigated with the effluent from Parkandabad wastewater treatment plant and the other one irrigated with well water over a period of six years in Iran. The results indicated that chlorine was increased significantly, in all depths (0-25, 25-50, 50-100, 100-150 and 150-200 cm) while exchangeable potassium, magnesium and phosphorous was increased significantly only in the top soil layer (0-25) and calcium content was increased up to depth of 50 cm. Irrigation with the treated wastewater increased soil Sodium content in all depths except for the depth of 100-150 cm but did not affect the soil nitrogen content significantly.

Mojiri (2011) carried out a greenhouse experiment to investigate the effects of municipal wastewater treatments on physical and chemical properties of saline soil Isfahan province (Iran) by irrigating with 100 ml, 200 ml and 300 ml of wastewater every day and a control having no wastewater irrigation. Soil irrigated with wastewater caused increase of EC, P, OM, TN, K, Na, Cl, Fe, Cd and Zn but it caused a decrease in soil pH. This result showed that soil irrigated with wastewater caused a decreased of Bulk density.

Magdaleno et al. (2011) investigated the concentration of heavy metals in agricultural soils and wastewater used for irrigation in plots of Mixquiahuala, Hidalgo having 80 years of irrigation history. In the wastewater the concentration of As, Cd, Hg, Ni, Cr and Zn was found within while the Pb concentration exceeded the maximum permissible limits in 40% of water samples analyzed while based on the limits established in Spain the concentrations of As, Ni and Cd exceeded permissible levels in 20, 60 and 60% respectively, of the samples analyzed. The concentration of extractable metals in agricultural soils was found in the following order: Pb > Ni > Cd > As > Cr > Hg.

**Objective: (2) To assess impact of wetland treated sewage water applications on the agricultural produce from sewage plot site**

Plants growth and yield are also affected by the presence of excess salts, heavy metals and pathogenic microbes in the wastewater. The excess specific ions like sodium, boron, chloride may cause phytotoxicity.

Irrigation water containing large amounts of sodium is absorbed by plant roots and transported to the leaves where it can accumulate and cause injury or may also

absorbed directly by plant leaves (in sprinkler irrigation) and will produce harmful effects.

The high chloride moves readily with the soil-water and taken up by the crop and accumulates in the leaves and may develop injury symptoms such as leaf burn or drying of leaf tissue first at the leaf tips (which is common for chloride toxicity), and progresses from the tip back along the edges as severity increases. Excessive necrosis (dead tissue) is often accompanied by early leaf drop or defoliation. The typical visible symptom of B toxicity is leaf burn chlorotic and/or necrotic patches, often at the margins and tips of older leaves (Bennett, 1993; Bergmann, 1992; Eaton, 1944). Plants are exposed to excess heavy metals in soil may accumulate and contaminate plant tissue or may also exhibit symptoms of phytotoxicity such as inhibition of seed germination, decreased plant height, decreased tillering, reduced root growth, decreased shoot growth, leaf chlorosis and necrosis, decreased chlorophyll metabolism, and lower fruit and grain yield. Such exposure may also sometimes lead to plant death. The estimated phytotoxic concentration of heavy metals in irrigation water are for Zn (2000  $\mu\text{g/L}$ ), Cu (200  $\mu\text{g/L}$ ), Fe (5000  $\mu\text{g/L}$ ), Mn (200  $\mu\text{g/L}$ ), Ni (200  $\mu\text{g/L}$ ), Pb (5000  $\mu\text{g/L}$ ) and Cd (10  $\mu\text{g/L}$ ), respectively (Pescod, 1992). The pathogenic microbes like nematodes may damage the plant parts or act as plant disease vector. The different studies done to assess the impact by wastewater use on different plants and in different plant parts are as follows:

Rattan et al. (2005) conducted a case study to assess the long-term effect of (untreated) sewage irrigations on the heavy metal content in the soils, plants (Cereals vegetables, fodder including paddy & wheat) and groundwater in the peri-urban agricultural lands under Keshopur Effluent Irrigation Scheme (KEIS) of Delhi, India. Along with soil they got significant accumulation of metal, in different agricultural produce. Rice grain accumulated much higher amount of Zn and Cu and a slight in Ni while their straw accumulated almost two times more Ni produced with sewage water over that of tubewell water irrigation. Wheat had elevated contents of Zn, Cu, Fe, Mn and Ni, Sorghum accumulated higher amount of Fe, Cu and Ni, Spinach accumulated higher amount of Zn, Cu and Ni, Maize showed higher accumulation of all the elements on sewage effluent irrigated soils. Similar trends were observed in gobhi sarson and oats with only exception of Mn. Cadmium & lead contents in tissues of all the crops were got below the detection limits. Concentration of metals in all the crops grown on sewage irrigated soils was below the level of phytotoxicity except Fe

contents in sorghum, maize, spinach, cucumber and Egyptian clover exceeded the phytotoxicity limit (>500 mg/kg).

The accumulation of heavy metals in dry matter content of rice grain & their straw, grown on the sewage irrigated soil was reported as follows: Zn (49.6 & 58.9 mg/kg), Cu (51.6 & 57.2 mg/kg), Fe (122 & 233 mg/kg), Mn (53.3 & 208 mg/kg) & Ni (10.1 & 10.4 mg/kg) while on tubewell irrigated soil Zn (29.6 & 61.9 mg/kg), Cu (23.0 & 59.8 mg/kg), Fe (186 & 202 mg/kg), Mn (88.1 & 229 mg/kg) & Ni ( 9.85 & 5.27 mg/kg), respectively.

The accumulation of metals in dry matter content of wheat grain grown on sewage irrigated soil was Zn (65.3 mg/kg), Cu (9.39 mg/kg), Fe (404 mg/kg), Mn (15.3 mg/kg) & Ni (20.0 mg/kg) while on tubewell water irrigated grain Zn (47.5 mg/kg), Cu (7.45 mg/kg), Fe (336 mg/kg), Mn (13.6 mg/kg) & Ni (19.7 mg/kg). Thus wheat had elevated content of all metals (Zn, Cu, Fe, Mn and Ni) compared to tubewell irrigation.

Jouzdani et al. (2007) conducted a lysimeter experiment cultivated with crops & vegetables with groundwater, untreated & treated urban effluent in Nashabia, Syria. The heavy metals (As, Cd, Cr and Pb) increased in fruits and leaves of the studied vegetables (Eggplant and Lettuce), as well as in the grain and straw of field crops (wheat and corn) grown by urban sewage treated effluent on lysimeter. The concentrations of Pb, Cr, Cd and As in plant tissues were found higher in the treatments irrigated with urban untreated effluents > treated > groundwater, in all plant tissues. The Cr concentration was higher than the normal concentration in Eggplant (green cover) but not in fruits for both treated and untreated effluent and within toxicity limit. However, with the exception of Cd, the content of As, Cr and Pb in the crops, was found within the acceptable natural range.

The accumulation of heavy metals in the dry matter content of wheat grains & their straw, grown by urban untreated effluent irrigation were found as follows: Pb (5.81 & 3.09 mg/kg), Cr (0.70 & 0.58 mg/kg), Cd (0.35 & 0.35 mg/kg) & As (0.19 & 0.17), while grown by urban treated effluent like Pb (5.22 & 2.13 mg/kg), Cr (0.57 & 0.40 mg/kg), Cd (0.34 & 0.29 mg/kg) & As (0.18 & 0.15 mg/kg), and in groundwater grown grains like Pb (3.44 & 0.29 mg/kg), Cr (0.17 & 0.15 mg/kg), Cd ( 0.28 & 0.26 mg/kg) & As (0.11 & 0.08 mg/kg), respectively.

Chung et al. (2011) conducted a pot experiment in a plastic film house in Korea to evaluate the translocation and uptake of heavy metals (Pb, Cd, Cu, and Zn)

into brown rice (*Oryza sativa* L.) and the heavy metals residues in soils by irrigated with domestic wastewater for a long time (3 years). The range of Pb, Cd, Cu and Zn was  $0.370 \pm 0.006$ ,  $0.011 \pm 0.001$ ,  $0.340 \pm 0.04$  and  $2.05 \pm 0.18$  mg/kg, respectively, in the domestic wastewater irrigated brown rice which indicates slight increased but lower than the recommended tolerable levels proposed by FAO/WHO.

Saha et al. (2010) conducted a field experiment to evaluate the impact of domestic sewage water on Vertisol & grown wheat crop at Bhopal, India. Bold seed size coupled with higher protein and Zn content in wheat grains showed an improved seed quality due to sewage irrigation. Nutrients supplied through sewage water were not found sufficient to raise the productivity of wheat to the level that obtained through fertilizers at the recommended level which indicated that additional nutrients particularly N through fertilizer are required to obtain higher productivity of wheat under sewage farming. Overall by municipal sewage water irrigation did not found any appreciable harmful effect rather increase wheat productivity, and produce quality.

At panicle initiation stage, flag leaves of wheat grown with sewage irrigation had significantly higher concentrations of N, P, Na, Cu and Fe, but lower concentration of Ca compared to that grown with groundwater. In wheat grains significant increase in N (correlated with the increase in protein content by 19%) and Zn concentration was also found due to sewage irrigation. Average grain and straw yield of the crop were significantly increased (31% and 43%, respectively) with sewage irrigation.

The grain yield, straw yield, 1000 seed weight, seed width, seed N content and seed Zn content of sewage irrigated wheat was found as 38.98 Mg/ha, 51.51 Mg/ha, 49.1 g, 3.11mm, 2.63% and 29.17  $\mu$ g/kg while the same on groundwater irrigated wheat was 29.77 Mg/kg, 35.99 Mg/kg, 45.1 g, 2.77 mm, 2.20% and 25.28  $\mu$ g/kg, respectively. The grain yield in sewage-irrigated control plot (receiving no fertilizers) was got almost same to the groundwater-irrigated plots receiving 50% of recommended general doses of NPK+FYM at 10 Mg/kg.

Singh et al. (2012) conducted an experiment for a year in Nagpur, India by growing wheat (AKW-1071), Gram (Jacky-9218), Palak (Pusa Jyoti), Methi (Kasuri) and Berseem (Multicut) with groundwater and domestic wastewater irrigation along with the recommended NPK dose of fertilizers. Use of the domestic waste water with fertilizers has shown the improvement in the physicochemical properties of the soil,

crop yield (both grains & straw) and also in the nutrient status as compared to that of the resulted from the application of groundwater with fertilizer. The domestic wastewater irrigation applied for a season had no significant effects apart from, slight changes in salt solubility and alkalinity on a clay soil with sewage wastewater irrigation.

The heavy metal content in sewage water grown wheat was Fe ( $25.71 \pm 1.10$ ), Mn ( $12.95 \pm 2.24$ ), Zn ( $16.90 \pm 3.28$ ), Cu ( $4.46 \pm 0.84$ ), Pb ( $3.89 \pm 0.56$ ) and Ni ( $2.75 \pm 0.26$  mg/kg), respectively, while the same in well-water grown wheat Fe ( $19.43 \pm 0.86$ ), Mn ( $10.36 \pm 1.56$ ), Zn ( $15.63 \pm 1.18$ ), Cu ( $3.75 \pm 0.26$ ), Pb ( $1.48 \pm 0.32$ ) and Ni ( $0.84 \pm 0.12$  mg/kg), respectively. The grain yield, straw yield and test weight (100 seed) of sewage grown wheat grains was found ( $17.37 \pm 0.22$  q/ha), ( $22.81 \pm 1.24$  q/ha) and ( $3.70 \pm 0.34$  g), respectively, while for well-water grown wheat ( $17.14 \pm 0.56$  q/ha), ( $22.33 \pm 3.36$  q/ha) and ( $3.53 \pm 0.72$  g), respectively. Among all crops wheat recorded highest yield.

Hussain et al. (2010) conducted a survey in Faisalabad, Pakistan where raw city effluent was used for irrigation from more than 25 years to appraise Cd concentration in these waters, soil and its uptake by cereal and legume crops as compared with the tube well and canal water irrigated areas. Seeds of effluent irrigated chickpea acquired the highest concentration of Cd (0.177 mg/kg), while was the lowest in wheat seeds (0.034 mg/kg). Concentration of Cd was higher in mungbean shoots (0.62 mg/kg) than in wheat shoots. The order for Cd concentration in seeds was chickpea (0.010-0.350) > maize (0.004-0.040) > mungbean (0.02-0.15) > wheat (0.034 mg/kg) for wastewater irrigated crops.

The mean cadmium concentration in wheat seeds, shoots and roots was 0.034, 0.123, 0.208 mg/kg at the effluent irrigated location while the corresponding values were 0.013, 0.019 and 0.208 mg/kg at canal irrigated sites and 0.010, 0.016 and 0.028 mg/kg in tube-well irrigated areas, respectively. Thus, effluent irrigated wheat accumulated 2.4, 7.2 and 7.3 times more Cd in seeds, shoots and roots than canal irrigated and 3.4 times more than tube-well irrigated plants.

Although Cd was in permissible limit for their use as irrigation, the effluent water was unsafe for irrigation. Plant roots retained most of the absorbed Cd from soil with little transfer to shoots and least into the seeds of all crops. It seems better to prefer cereal cultivation over leguminous crops on effluent irrigated soils to reduce the hazard of Cd contamination.

Minhas et al. (2006) conducted an experiment at the research farm of Central Soil Salinity Research Institute at Karnal where different cropping systems viz. vegetable (cabbage–ridge gourd), forage (sorghum–Egyptian clover), grain (paddy–wheat) and agro-forestry (paddy–wheat with poplar) were being irrigated with sewage water having the faecal coliform counts averaged  $1.5 \times 10^8/100$  ml. Edible parts of different crops were collected and analyzed for total bacterial counts, Faecal coliform, fungi, Salmonella and Shigella. The aerobic bacterial plate counts for vegetables, fodder and grain crops ranged between  $2 \times 10^6$  and  $3.5 \times 10^7$ ,  $6 \times 10^6$  and  $3 \times 10^8$ ,  $2 \times 10^5$  and  $3.8 \times 10^{10}$ , respectively, while the corresponding Faecal coliform ranged between  $< 2$  and  $9 \times 10^5$ ,  $9 \times 10^2$  and  $2 \times 10^5$  and  $< 2$ , indicating that the pathogenic loads in the produce. The average bacterial count was found  $3.8 \times 10^{10}$  in wheat and  $2 \times 10^5$  in paddy grains while MPN count of Faecal coliform  $< 2/100$ g in both wheat and paddy. But, the contamination was got below permissible level in the produce that was harvested after sun drying in the field itself, whereas the parts coming in direct contact were the most severely contaminated.

Gupta et al. (2009) conducted a study in Titagarh a suburban industrial town in West Bengal where farmers use both treated and untreated sewage effluent for growing vegetables.

A total of 46 wastewater samples (untreated: 24 and treated: 22), 35 soil samples and 172 vegetable samples were collected from the wastewater-irrigated area of Titagarh to assess its contamination level with intestinal helminth. 83.3% of raw wastewater, 68.2% of treated wastewater, 68.6% of soil and 44.2% of vegetables in the study area were found to be positive for helminth ova. Vegetables grown in this area were found positive for *Ascaris lumbricoides* (36%), *Trichuris trichiura* (1.7%) and hookworms (6.4%). *A. lumbricoides* was the most predominant species observed in all the samples. Of all the vegetables examined, Pudina was most commonly contaminated followed by Lettuce, Spinach, Coriander, Celery and Parsley.

Yadav et al. (2002) conducted a study in Kurukshetra, Haryana (India) to assess the impact of about three decades of irrigation with domestic sewage effluent on alluvial soil & their effects on the composition of crops and ground waters. Some changes in nutrient contents of soils also reflected in uptake by winter (wheat, berseem) and summer (rice, sorghum) crops growing at these sites. Higher contents of N, P, K and Na were monitored in their shoots when grown on soils irrigated with sewage water. Higher accumulation of micro-nutrients and heavy metals in plants

was also observed in sewage water irrigated crops, but by and large the contents of Pb, Cd, Zn, Mn, Cu, Fe and Ni were within the critical limit. There was constant decline in their concentrations with distance of sampling from the disposal point. Municipal sewage used for about three decades showed the enrichment of soils with both organic matter and nutrients without excessive accumulation of any toxic elements in soils and plants. However, traces of some of the toxic ions like Ni, Cd and Pb were noticed in plants and that  $\text{NO}_3^-$  in some well waters should be a matter of concern and indicate the need for continued monitoring or treatment of sewage water before it is let into disposal channel for irrigation.

Yoon and Kwun (2001) performed a pilot study in an experimental field at Konkuk University in Seoul, Korea, to examine the effect of constructed wetland treated sewage irrigation on paddy rice culture and its soil characteristics. Treated sewage irrigation resulted in about 10% (with dilution) or 50% (without dilution) greater yield than in controls. The strength of treated sewage did not give any adverse effect on paddy soil, plant growth and no lodging was observed in the rice culture even with a relatively high nitrogen concentration (up to 160 mg/l). Although there was an indication of salt accumulation in the field irrigated with the highest strength of treated sewage was observed. But overall, treated sewage could be reused as a supplemental source of irrigation water for paddy rice culture without causing adverse effects as long as it is treated adequately and used properly.

Feizi (2001) conducted a study in Isfahan city (Iran) where farmers were growing wheat, corn, tomatoes, cucumber and alfalfa with “wells treated wastewater” for about 8 years and compared them with the fields irrigated with well water. In general, concentration of Zn, Mn, Cu and Fe in soil was higher in fields irrigated with effluent, with Mn and Zn more significant. The amount of Fe and Mn in corn (forage), Mn and Zn in the straw & grain of wheat, Mn, Zn and Cu in tomato stems & leaves while Fe, Zn and Cu in tomato fruit, Fe, Mn, Zn and Cu (mainly Fe and Cu) in cucumber (fruit) were significantly higher whereas in alfalfa (forage) except Fe and Zn the accumulation of elements were not significant in effluent irrigated fields, than well water irrigated. In overall plant analysis, only the amount of Fe was higher than critical level for both irrigation types. In general, the heavy metal concentration in the soil and plants were slightly higher in irrigation with effluent, but there appears to be no significant risk associated with the irrigation of the crops with treated wastewater in relation with the heavy metals.

Kalavrouziotis et al. (2008) conducted a greenhouse experiment in Agrinion, Greece; to assess the effect of treated municipal wastewater (TMWW) in *B. oleracea* var. *Italica* (Broccoli), and *B. oleracea* var. *Gemmifera* (Brussels sprouts), as well as on the physical and chemical properties of the clay loam soil. They found significant increase in the heavy metal content in the roots (dry matter) like in Brussel sprout Cd (0.0083 - 0.78), Co (0.029 - 3.38), Ni (4.83 - 7.27) and Fe (379.5 to 1022.0 mg/kg), respectively and in Broccoli Ni (4.20 - 10.13 mg/g). However, the levels of the heavy metals in the edible plant parts (heads and sprouts) were also very high like in Broccoli Ni (3.91–4.15 mg/g) and Pb (9.82–10.40 mg/g), while in Brussels sprouts Cd (0.8–1.17 mg/g), Co (2.35–2.70 mg/g) and Ni (5.70–6.17 mg/g) which may cause health risk after consumption.

Khan et al. (2011) conducted a field experiment near Palosi drain, Peshawar (Pakistan) to study the effect of tube well (TW) and wastewater (WW) with or without basal dose of fertilizers on the yield and heavy metal uptake of tomato. Wastewater irrigated plants found taller and having higher biomass in both with or without fertilizers. The results showed that leaves accumulated higher concentration (with exception of Cu) of heavy metals than fruit. The concentration of Cr, Fe, Mn Pb and Zn in leaves & the concentration of Fe and Pb in fruits was found above the permissible limits indicating toxicity in wastewater irrigated plants. The plants receiving sole application of WW accumulated more heavy metals compared to WW plus half dose of Fertilizer (NPK). It was concluded that tomato can be irrigated with effluents containing moderate supply of heavy metals on coarse textured soil.

Banania et al. (2010) conducted a field experiment in non-agricultural soil to investigate the effect of treated municipal wastewater (TMW) on the yield and fiber quality of cotton (*Gossypium hirsutum* L.) in Iran. The treatments were irrigated with surface irrigation by different mixtures and intervals of freshwater and TMW. Two additional treatments irrigated with freshwater and TMW were considered as control. The results showed that cotton yield, number of bolls per m<sup>2</sup>, leaf area index (LAI) and plant height were significantly greater with TMW irrigation rather than with freshwater irrigation. The yield of TMW and freshwater treatment was about 2200 and 780 kg lint/ha, respectively. There is no significant effect between interval and mixture treatment with the freshwater and TMW application. Also the TMW had no significant detrimental effect on the characteristics of cotton fiber quality.

Rebora et al. (2010) compared the effect of Urban wastewater (UWW) irrigation on energy crops Jerusalem artichoke (*Helianthus tuberosus* L.) and “Gospel” winter rape cultivar (*Brassica napus* L.) in Mendoza province (Argentina) as compared to groundwater (GW).

In case of Jerusalem artichoke they found higher average tuber yield in UWW (177,750 kg/ha) compared to GW (144,000 kg/ha), the average number of tubers produced per plant in UWW (108.4) compared to GW (89.58), the average plant height in UWW (2.97) compared to GW (2.71m), number of stem per plant in UWW (1.54) compared to GW (1.35), the average dry aerial biomass per plant in UWW (0.70 kg) compared to GW (0.60 kg), the remanent dry aerial biomass of the crop per ha in UWW (23,250 kg) compared to GW (17,750 kg). Thus the potential to produce ethanol was 15,000 l/ha in plots irrigated with UWW and 13,000 l/ha in the GW irrigated.

In case of rapeseed the average number of plant per plot was lower in UWW (27.5) and GW (33.32) but, the average number of siliqua per plant was found 692.3 in UWW plots compared to 533.7 in GW plots, the average number of seeds per siliqua in UWW plots (23.07) compared to (GW 20.48), the average one thousand seeds weight in UWW (3.60) compared with GW (2.43), the yield in UWW plots (7,690 kg/ hectare) compared to GW plots (3,886 kg/ hectare), Oil percentage found in seeds of both treatments was lower than that indicated for Gospel cultivar (47%) in UWW (36.7%) compared to GW seeds (36.2%). Thus, the potential to produce biodiesel per unit surface was 2,822 L biodiesel/hectare in when rape is irrigated with urban waste-water and 1,406 litres biodiesel/ha when it is irrigated with ground water.

Gharsallaoui et al. (2011) conducted an experiment in El Hajeb (Tunisia) to determine the impact of wastewater irrigation on the quality of the oil. The results obtained showed that olive trees benefitted from this contribution of wastewater, irrigation by wastewater has a significant effect in the fatty acid composition like increasing the percentage of palmitoleic acid, linoleic (C18:2) and linolenic (C18:3) and decreases the percentages of stearic and oleic acid. The oils irrigated with wastewater are more sensitive to the oxidation especially when olives are stored before the extraction, oils coming from olive trees irrigated with wastewaters are richer in polyphenols and the fallen olives collected from the ground irrigated with wastewaters provide poor quality oils.

Arora et al. (2008) carried out a study to assess levels of different heavy metals in vegetables irrigated with water from different sources. The results indicated a substantial build-up of heavy metals in vegetables irrigated with continuous long term irrigation with wastewater like 116–378, 12–69, 5.2–16.8 and 22–46 mg/kg for iron, manganese, copper and zinc, respectively. The highest mean levels of Fe and Mn were detected in mint (378 mg/kg and 67 mg/kg) and spinach (309 mg/kg and 69.4 mg/kg), whereas the levels of Cu (16.8 mg/kg) and Zn (46.4 mg/kg) were highest in carrot. Wastewater-irrigated spinach has shown significantly higher accumulation of Fe, Mn, Cu and Zn, compared to the freshwater-irrigated spinach, indicating the highest metal absorption for this vegetable and not suitable for consumption. All the vegetables contained heavy metals were lower than the recommended tolerable levels proposed by Joint FAO/WHO Expert Committee on Food Additives.

Akponikpè et al. (2011) evaluated the physico-chemical and biological risks on irrigated soils and fruits of macrophyte treated wastewater (TWW), the nutrients supply, and the effect on tomato and eggplant production in semi-arid Burkina Faso, Vezina (West Africa). The study revealed that the treated wastewater without mineral fertilizer improved eggplant yield (40% in average) compared to the freshwater. Both crops responded better to mineral fertilizer (52% for tomato and 82% for eggplant) and the effects of the treated wastewater and fertilizer were additive. As the N supply of TWW was very unsteady (8–227% of crop need), and P<sub>2</sub>O<sub>5</sub> supply did not satisfy in whole crop need (3–58%), but the K<sub>2</sub>O supply was more steady and close to crop requirement (78–126%) over the three years of experiment and no addition of K fertilizer needed. Faecal coliforms and helminth eggs were observed in treated wastewater and irrigated soils at rate over the FAO and WHO recommended limits for vegetable to be eaten uncooked. Tomato fruits were observed to be faecal coliform contaminated with treated wastewater.

Surdyk et al. (2010) studied the accumulation of heavy metals in soil and potato plants (*Solanum tuberosum* L.) irrigated with treated low quality surface water in a three years experiment in north of Belgrade, Serbia. Irrigation water was treated using (1) a conventional sand filter treatment, and (2) a sand filter combined with a specific filter for heavy metal removal treatment. In spite of this variability of the irrigation water composition, the soil contents in inorganic elements at the end of the three irrigation years are similar to the initial state. After the third harvest, no impact of the irrigation water on potato quality could be detected except for total sugar and

sugar in total solids. It was cleared that appropriately treated, low quality feed waters with high heavy metal contents can be used for irrigation over several years without significant degradation of soil and produces.

Mukherjee and Mishra (2008) conducted a study to investigate the level of heavy metal in sewage water, soil and some vegetables like spinach (*Spinach oleraceae*) and cauliflower (*Brassica oleracea* var. botrytis) grown in STP area Bhagwanpur (Varanasi) where farmers use treated sewage for irrigation for the last two decades. Heavy metals in irrigation water were higher than the recommended level and the heavy metal concentration in soil was below the Indian standard for all heavy metals except for Cr in summer. In edible portion of spinach Cd, Cu and Cr concentration (5.93, 28.15 and 12.02 mg/kg) while in cauliflower Cr, and Cd (5.95 and 1.96 mg/kg), respectively, in Madarva was higher than the permissible limits and may causing potential health risk in the long term.

Varalakshmi and Ganeshamurthy (2010) conducted a study in peri-urban Bangalore where city wastewaters from four water bodies viz, Bellandur, Varthur, Byramangala and Nagavara tanks were used for cultivation of vegetable crops to assess heavy metal contamination of water, soil and vegetables. Analysis revealed high concentrations of Cd and Cr in waters of all the tanks, exceeding the recommended levels. Concentration of Cd was highest in waters of Bellandur (0.039mg/L) and concentration of Cr was highest in waters of Byramangala tank (0.311mg/L). Among all the tanks, Bellandur and Varthur were found to be highly contaminated with Cd, Pb and Ni. The concentration of heavy metals (mg/kg) in soils receiving sewage waters from the four tanks ranged from 1.92 -2.90 for Cd, 47.04-68.12 for Pb, 35.08-92.78 for Cr and 48.2-57.3 for Ni. The Cd and Pb contents were highest in the soils near Varthur and Bellandur tanks, while Cr was highest in soils near Byramangala. A similar trend was observed with respect to heavy metal content of vegetables. Among all the vegetables, Amaranthus and palak accumulated higher concentrations of heavy metals followed by carrot and radish. The Cd concentration of all the vegetables grown near Varthur and Bellandur tanks exceeded the PFA safe limit. Pb and Ni concentrations exceeded the safe limits in all the vegetables in all the tank areas.

Kiziloglu et al. (2008) conducted a field experiment to investigate the effects of irrigation with untreated, and preliminary and primary treated wastewater on macro and micronutrient distribution within the soil profile, yield and mineral content of

cauliflower and red cabbage plants in eastern Anatolia, Erzurum province, Turkey. Wastewater irrigation affected significantly soil chemical properties in the 0–30 cm soil layer and plant nutrient content after harvest. Application of wastewater increased soil salinity, organic matter, exchangeable Na, K, Ca, Mg, plant available phosphorus and microelements, and decreased soil pH. Wastewater irrigation treatments also increased the yield as well as N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, Pb, Ni and Cd contents of cauliflower and red cabbage plants. The highest yield, macro- and micronutrient uptake of cauliflower and red cabbage plants were obtained with the untreated wastewater. Heavy metal contamination in soil and plant, and salinity were not observed with the application of wastewater. It can be concluded that untreated wastewater can be used confidently, in the short term, in agricultural land, while primary treated wastewater can be used in sustainable agriculture in the long term.

**Objective: (3) To assess comprehensive risk of untreated and treated sewage water use on the consumer health**

Comprehensive risk assessment of treated wastewater is important due to the presence of extensive amount of heavy metals in the wastewater which may harm to soil through successive accumulation after long term continuous use, affect groundwater through leaching and entire food chain through translocation in the agricultural produce. Consumption of these contaminated produce may also affect the human health. Risk assessment is done with the help of some indices or health based indicators which are described in the section-2. Different researchers use these indices to assess the risk for different conditions which are as follows:

Liu et al. (2005) conducted a case study to assess the impacts of sewage irrigation on soil heavy metal content in Beijing, China. Concentrations of Cd, Cr, Cu, Zn, and Pb were determined in samples and compared with the reference values of 1970s, the pollution load index (PLI), enrichment factor (EF), and contamination factor (CF) of these metals were calculated. The pollution load indices (sewage irrigation land 3.49) of soils indicated that metal contamination occurred in these sites. The metal enrichment (EF of Cd 1.8, Cr 1.7, Cu 2.3, Zn 2.0, Pb 1.9) and the metal contamination (CF of Cd 2.6, Cr 1.5, Cu 2.0, Zn 1.7, Pb 1.6) showed that the accumulation trend of the five toxic metals increased during the sewage irrigation as compared with the lower reference values than other region in China and world average. The EF for Cu (2.31) was the highest, but the actual concentration was not

much higher than the normal requirement because Cu is essential for plants. However, the CF for Cd (2.6) was the highest value for the five heavy metals followed by Cu. The TF values for Cd (0.17 - 4.06) varied greatly between crops, and they were generally higher than those for the other four metals which was highest for long leaf lettuce (4.06) while lowest in Rice grain (0.17), followed by those for Zn (0.42 - 0.95) and Cu (0.15-0.86). The TF for Zn from soil to vegetable was the highest in spinach (0.95). The TF values for Pb (0.12-0.22) are similar to those for Cr (0.01-0.19). The high TF values for Cd and Zn from soil to crop indicate a strong accumulation of Cd and Zn by crops. In Rice grain the observed TF value was for Cd (0.17), Cr (0.01), Cu (0.20), Zn (0.42) and for Pb (0.12).

Wang et al. (2009) studied the accumulation and bioavailability of copper and nickel in wheat plants grown in contaminated soils from the oasis, northwest China. The concentrations of Cu and Ni in studied soils were more than Cu and Ni limit of grade II and grade III soil environmental quality standards of China.

The sequences of Ni average concentrations in different parts of the wheat plant were roots (59.6 mg/kg) > leaves (46.7 mg/kg) > shells (21.9 mg/kg) > stocks (4.12 mg/kg) > grains (3.75 mg/kg); while the sequences of Cu average concentrations in different parts were roots (67.8 mg/kg) > leaves (61.7 mg/kg) > shells (27.1 mg/kg) > grains (9.69 mg/kg) > stocks (6.70 mg/kg). Thus, soil was not fit to plant the wheat crops for human consumption and posed high potential health risk for human health through food chains.

The order of average value of Bio-concentration factor (BCF) of Cu in the different parts of wheat plants were root (0.2449) > leaves (0.2229) > shell (0.0977) > grain (0.0350) > stock (0.0242); while of Ni were root (0.1986) > leaves (0.1560) > shell (0.0729) > stock (0.0137) > grain (0.0125). Thus, BCF indicated that copper and nickel could accumulate in the roots and leaves of wheat plants in higher amounts than other parts of wheat plants. Average BCF of Cu and Ni in grains was 0.035 and 0.013, respectively i.e. only 3.5% and 1.3% of soil Cu and Ni enter into grains, respectively.

The order of average value of Transfer factor (TF) of Cu from root to other parts of wheat plants were leaves (0.9101) > shell (0.3988) > grain (0.1429) > stock (0.0987); while of Ni leaves (0.7856) > shell (0.3673) > stock (0.0688) > grain (0.0629). Thus, there was a large difference for concentration of copper and nickel between the root and other parts indicated a restriction of the internal transport of

copper and nickel from root to other parts of wheat plants. The roots have strong capability to hold copper and nickel against the transport to other parts of wheat plants. In general, TF of Cu is greater than Ni in different parts of wheat plants. Grains contained 9–26% and 2–16% of Cu and Ni found in roots, respectively. Average TF of Cu and Ni in the grain was 14% and 6%, respectively.

Average Bio-concentration factor (BCF) and translocation factor (TF) of Cu in different parts of wheat plants were greater than those of Ni. The process of Cu and Ni uptake and accumulation by wheat plants were probably linked to the concentrations of available copper and nickel in the soils, physical and chemical parameters of soils, and different parts of wheat plants. Only small amount of Cu and Ni is accumulated in the grains.

Rattan et al. (2005) conducted a case study to assess the long-term effect of (untreated) sewage irrigations on the heavy metal content in the soils, plants and groundwater in the peri-urban agricultural lands under Keshopur Effluent Irrigation Scheme (KEIS) of Delhi, India. It was found a substantial build-up of Zn, Cu and Ni in the bio-available pool of sewage-irrigated soils. In case of Zn and Fe, TFs for all the crops grown on sewage effluent irrigated soils were lower than that for crops produced with tubewell water irrigation. However, for other metals, such consistent variations in TFs were not obtained. Although long-term sewage irrigation resulted into elevated concentration of metal in soil, the same would not be proportionately transferred to food chain. Taking all the crops together, relative orders of transfer of metals from soil to plants grown on sewage irrigated soils were  $\text{Ni} > \text{Zn} > \text{Fe} > \text{Mn} > \text{Cu}$ . These results show that as far as entry of these metals to food chain plants is concerned, Ni has the greatest potential, followed by Zn, Fe, Mn and Cu.

Based on the soil to plant transfer ratio (transfer factor) of metals, relative efficiency of cereals, millet and vegetable crops to absorb metals from sewage and tubewell water-irrigated soils were analyzed and risk assessment to human health with respect of metal contents were done in terms of hazard quotient. The values of Hazard quotient of green vegetables (HQ<sub>gv</sub>) for gobhi sarson varied from 0.040 to 0.068 for Zn, 0.004 to 0.021 for Cu and 0.027 to 0.442 for Ni. In case of spinach, HQ<sub>gv</sub> varied from 0.035 to 0.152, 0.008 to 0.015 and 0.046 to 0.502 for Zn, Cu and Ni, respectively. The values of HQ<sub>gv</sub> for Indian rape ranged from 0.027 to 0.053, 0.004 to 0.014 and 0.016 to 0.429 for Zn, Cu and Ni, respectively. Although, Ni exhibited relatively higher HQ for all the crops compared to other two metals, most of the

values were far less than 1. Hence, these green vegetables are not likely to induce any health hazard to consumers (human) as far as its metal contents are concerned.

Singh et al. (2010) conducted a study around Dinapur sewage treatment plant (DSTP) a suburban area in the north east of Varanasi where farmers use industrial effluent rich treated and untreated wastewater for large-scale vegetable production and supplied to the markets. They assess the risk to human health by heavy metals (Cd, Cu, Pb, Zn, Ni and Cr) through the intake of locally grown vegetables, cereal crops and milk from wastewater irrigated site.

Heavy metal concentrations were several fold higher in all the collected samples from wastewater irrigated site compared to clean water irrigated ones. Cd, Pb and Ni concentrations were above the 'safe' limits of Indian and WHO/FAO standards in all the vegetables and cereals, but within the permissible limits in milk samples.

Within the plants, cabbage (leafy vegetable) showed highest Enrichment factor (EF) value (10.88) for Cd and amaranthus (20.69) for Ni. EF of other metals like Pb, Zn and Cr was highest in pumpkin (52.12), lady's fingers (6.14) and rice (31.51), respectively. EF value in Wheat for Cd (4.57), Cu (1.54), Pb (34.98), Zn (2.42), Ni (15.26) and Cr (0.58), while in Rice for Cd (6.87), Cu (1.23), Pb (31.59), Zn (1.52), Ni (7.98) and Cr (31.51), respectively.

Among different vegetables, cabbage (11.82) showed highest value of MPI followed by palak (11.54). As compared to the vegetables, wheat (6.92) and rice (7.29) showed lower metal pollution index. Higher MPI of cabbage (11.82), palak (11.54), brinjal (10.38) and lady's finger (11.35) suggests that these vegetables may cause more human health risk due to higher accumulation of heavy metals in the edible portion.

To assess the health risk associated with heavy metal contaminated produce Health risk index (HRI) were calculated. The results showed that Cd, Pb and Ni contamination in plants had greatest potential to pose health risk to the consumers. HRI was found more than 1 for Cd in all the plants except radish, pointed gourd and tomato. For Pb, it was higher in all the leafy vegetables, lady's finger, brinjal, bottle gourd and cereal crops, whereas for Ni, it was higher in cereal crops and all the leafy vegetables except cabbage. HRI value was found in Wheat for Cd (5.86), Cu (5.16E-02), Pb (4.36), Zn (4.81E-03), Ni (2.40) and Cr (2.97E-04) while in Rice for Cd (9.15), Cu (4.39E-02), Pb (6.83), Zn (8.40E-03), Ni (1.32) and Cr (2.17E-04),

respectively. In the present study, Cu, Zn and Cr were not found to cause any risk to the local population.

Cadmium, Pb and Ni concentrations were above the national and various international permissible limits in all the vegetables and cereal crops. The metal pollution index and health risk index of heavy metals also suggest that Cd, Pb and Ni contamination in most of the test plants had potential for human health risk due to consumption of plants grown at waste water irrigated site. Milk is found to be least contaminated by heavy metals as its metal pollution index and health risk index were lower compared to other foodstuffs. The study suggests that even though there are low concentrations of heavy metals in irrigation water, its long term use caused heavy metal contamination leading to health risk of consumers. Thus urgent attention is needed to devise and implement appropriate means of monitoring and regulating industrial and domestic effluent.

Liu et al. (2011) assessed the impact of long-term electroplating industrial activities on heavy metal contamination in agricultural soils and potential health risks for local residents. Fractionation and risk assessment code (RAC) were used to evaluate the environmental risks of heavy metals in soils. The health risk index (HRI) and hazard index (HI) were calculated to assess potential health risks to local populations through rice consumption. Hazardous levels of Cu, Cr, and Ni were observed in water and paddy soils at sites near the plant. According to the RAC analysis, the soils showed a high risk for Ni and a medium risk for Cu and Cr at certain sites. The rice samples were found contaminated with Ni, followed by Cr and Cu. HRI values  $>1$  were not found for any heavy metal. The HRI of heavy metals from rice consumption is in decreasing order:  $\text{Cu} > \text{Ni} > \text{Pb} > \text{Cd} > \text{Cr}$  which suggested that Cu ingestion has the highest potential health risk and Cr ingestion has minimum risk. However, HI values for adults and children were 2.075 and 1.808, respectively, indicating that both adults and children may experience some adverse health effects. The relative contributions of Cu, Cr, Ni, Pb, and Cd to the HI are 41.0%, 0.2%, 32.1%, 18.1%, and 8.6%, respectively. Therefore, Cu and Ni are key components contributing to the potential health risk of non-carcinogenic effects for adults and children, with Pb and Cd being second and Cr being the least contributing. The concentrations of Cu, Cr, and Ni in rice grains from downstream sites were higher than those from upstream sites, indicating that contamination of the rice samples were related to the electroplating wastewater. Although no single metal poses

health risks for local residents through rice consumption, the combination of several metals may threaten the health of local residents. Cu and Ni are the key components contributing to the potential health risks.

Bo et al. (2009) conducted a survey for assessing the potential health risk to local inhabitants by heavy metals (As, Cd, Cr, Cu, Ni, Pb and Zn) concentrations in vegetables in Beijing. They collected 416 samples (involving 100 varieties) from different markets and divided in two groups open field vegetables and greenhouse vegetables.

The results indicated that the metal concentrations in vegetables ranged from < 0.001 to 0.479  $\mu\text{g/g}$  fresh weight (fw) (As), < 0.001 to 0.101  $\mu\text{g/g}$  fw (Cd), < 0.001 to 1.04  $\mu\text{g/g}$  fw (Cr), 0.024 to 8.25  $\mu\text{g/g}$  fw (Cu), 0.001 to 1.689  $\mu\text{g/g}$  fw (Ni), < 0.001 to 0.655  $\mu\text{g/g}$  fw (Pb) and 0.01 to 25.6  $\mu\text{g/g}$  fw (Zn), with average concentrations of 0.013, 0.010, 0.023, 0.51, 0.053, 0.046 and 2.55  $\mu\text{g/g}$  fw, respectively. The concentrations of As, Cr, Cu, Cd, Pb and Ni in vegetables from open-fields were all significantly higher than those grown in greenhouses. All elements except Zn in open-field vegetable and local-produced vegetable were all significantly higher than those in greenhouse vegetable and non-locally produced vegetable, respectively.

The estimated daily intake (EDI) of As, Cd, Cr, Cu, Ni, Pb and Zn from vegetables was 0.080, 0.062, 0.142, 3.14, 0.327, 0.283 and 15.7  $\mu\text{g}/(\text{kg bw-d})$  for adults, 0.102, 0.079, 0.181, 4.01, 0.416, 0.361 and 20.0  $\mu\text{g}/(\text{kg bw-d})$  for children, and 0.069, 0.053, 0.122, 2.70, 0.281, 0.245 and 13.5  $\mu\text{g}/(\text{kg bw-d})$  for seniors, respectively. Arsenic was the major risk contributor for inhabitants. The target hazard quotient (THQ) based on the weighted average concentration (THQw) of As amounted to 44.3% of the total THQ (TTHQ) value according to average vegetable consumption and the THQ based on percentile concentration (THQc) of As at the 85th percentile was close to or exceeded the acceptable safety value of unity for all age groups. The TTHQ was lower than 1 for all age groups, indicating that it was still safe for the general population of Beijing to consume vegetables.

Cao et al. (2010) investigated heavy metal (Cu, Zn, Pb, Cr, Hg and Cd) concentrations in rice, garden vegetables and cultivated soils, in a rural-industrial developed region in southern Jiangsu, China, and estimated the potential health risks of metals to the inhabitants via consumption of locally produced rice and garden vegetables. Rice and vegetables were accounted for 64% of total foodstuffs consumed, and over 60% were grown in the local region.

The proportion of cultivated soils with concentrations exceeding the corresponding maximum allowable concentrations (MACs) decreased in the order: Hg > Cr > Cu > Pb = Cd. Average concentrations of Cr, Cd and Pb in cultivated soils of both rice and garden vegetables were above the background values in soil of Jiangsu Province. Average concentrations of Cr, Zn and Hg in cultivated soils were above the national background values for soil with pH < 6.5.

Average concentrations of Cr, Cu, Zn, Cd, Hg and Pb were 0.75, 2.64, 12.00, 0.014, 0.006 and 0.054 mg/kg dw (dry weight) in rice and were 0.67, 1.18, 4.34, 0.011, 0.002 and 0.058 mg/kg fw (fresh weight) in garden vegetables, respectively, which were all below the MAC in food in China except for Cr in vegetables. Leafy vegetables had higher metal concentrations than solanaceae vegetables.

Average daily intake of Cr, Cu, Zn, Cd, Hg and Pb through the consumption of rice and garden vegetables were 5.66, 16.90, 74.21, 0.10, 0.04 and 0.43  $\mu\text{g}/(\text{kg}\cdot\text{day})$ , respectively. Although Hazard Quotient values of individual metals were all lower than 1, when all six metal intakes via self-planted rice and garden vegetables were combined, the Hazard Index value was close to 1. Cu, Zn and Pb were the top three metals with higher health risks. Potential health risks from exposure to self-planted rice and garden vegetables were of major concern. Health risks of metals induced by rice ingestion were higher than those induced by intake of garden vegetables in the studied area.

Harmanescu et al. (2011) conducted a study in Banat County (Romania) to measure the levels of heavy metals (Fe, Mn, Zn, Cu, Ni, Cd and Pb) in common vegetables (parsley, carrot, onion, lettuce, cucumber and green beans) grown in contaminated mining areas compared with those grown in reference clear area and to determine their potential detrimental effects via daily metal intake ( $DI_{\text{metal}}$ ) and Target Hazard Quotients (THQ) for normal daily consumption of these vegetables, for male and female gender.

Compared with the reference in contaminated areas, soil and plant contents of all analyzed metals (Mn, Zn, Cu, Cd and Pb) were higher. Particularly, in soil, higher values than intervention threshold values (ITV) were found for Cu and Pb and higher than maximum allowable limits (MAL) for Zn, Cu, Cd and Pb for parsley roots and leaves, carrot roots, cabbage, lettuce and cucumber.

The combined THQ values calculated were below the safe level of THQ < 1 for all vegetables grown in reference area. In contaminated Moldova Noua (M) area

the combined THQ exceeded the safe level only for parsley roots, while in more contaminated Ruschita (R) area combined THQ exceeded the safe level for parsley and carrot roots, lettuce and cabbage. Cd and Pb, most toxic metals to humans, have an increasing prevalence in the combined THQ for leafy (cabbage and lettuce) and fruit vegetables (cucumber). In the root vegetables only Pb had an increasing prevalence in combined THQ values. In all areas female THQ was higher than male THQ. Thus, the consumption of some vegetables (especially parsley, carrot and cabbage and less for lettuce, cucumber and green beans) is not free of risks in these areas.

Khillare et al. (2012) conducted a study by growing different vegetables (Radish, spinach, cowpea, bottle gourd, bitter gourd, ridge gourd) in the vicinity of three thermal power plants and a background site in Delhi, India and were analyzed for 16 priority polycyclic aromatic hydrocarbons (PAHs) and 7 heavy metals (Cd, Cu, Cr, Ni, Zn, Fe and Mn).

Annual mean concentrations of PAHs and all metals were found to be significantly higher (ANOVA,  $p < 0.001$ ) at power plant sites as compared to the background site. Higher abundances of coal tracer PAHs such as Phen, Anth, Flan, Pyr and Chry in power plant vegetables suggested possible impacts of coal combustion emissions. Comparatively higher ratios of polluted sample-to-background sample of vegetables were found especially for Cr, Cd, Ni and Zn as compared to Fe, Mn and Cu. Spinach and radish showed greater accumulation of PAHs and metals on a mass basis at the power plant sites while vegetables belonging to the gourd family showed highest relative enrichment. Pollutant distributions in vegetables were found to be influenced by seasonal conditions. Vegetables with considerable leafy parts, such as spinach and radish, were found to be more contaminated with respect to both PAHs and metals suggesting foliar intake as a major source.

Power plant samples showed 184–475% greater metal pollution index (MPI) values as compared to the background location while health risk indices (HRI) for Cd and Ni exceeded the safe limit for most vegetables. Incremental lifetime cancer risk (ILCR) assessment showed that up to 58 excess cancer cases are likely in Delhi for lifetime ingestion exposure to PAHs at their observed concentrations. Overall, the results of this study indicate a definite impact of power plant emissions on vegetables grown in the vicinity and the associated health risks.

Si et al. (2011) conducted a study to assess the efficiency of subsurface flow constructed wetland (CW) planted with *Typha angustifolia* in reducing the heavy metal contamination in irrigation water and food crops and the associated risk was assessed using hazard quotient (HQ) and hazard index (HI) in Silong town, China by growing wheat irrigating with untreated Yellow River (YW) water on plot A and CW treated YR water on plot B.

The results showed a potential health risk to inhabitants via consumption of wheat grain irrigated with untreated water from Yellow river (YR), while the health risk from the potential HMs toxicity was greatly reduced (35.19%) through CW pre-treatment of irrigation water which significantly decreasing the concentrations of HMs in wheat grain.

The trend of concentrations of four HMs in wheat grain in plot A, irrigated with YR water, was in the order of Zn>Cu>Pb>Cd. Of these HMs, Pb was higher than the maximum safe intake level and hygienic standard for grain. Through irrigating with CW treated water, the concentrations of Cu, Zn, Pb and Cd in wheat grain in plot B were significantly lower than that in plot A. The rates of grain HMs reduction reached 2.46 to 1.12 mg/kg (54.5%) for Cu, 15.6 to 7.77 mg/kg (50.2%) for Zn, 0.47 to 0.13 mg/kg (72.3%) for Pb and 0.03 to 0.01 mg/kg (66.6%) for Cd by CW treatment of YR water.

The high BCF values of Cu (0.08), Zn (0.19), Pb (0.02) and Cd (0.1) in wheat grain in plot A indicate a strong accumulation of the respective HMs by wheat grain while the CW had greatly decreased BCF values of Cu (0.04), Zn (0.16), Pb (0.01) and Cd (0.07), respectively.

The order of HQ was Pb>Cu>Zn>Cd. The HQ of Pb for children and adult males were above 1 in plot A, while HQ of each metal for different groups of people were all below 1 in plot B. Indicating that the consumption of wheat grain from plot A have a potential health risk to children and adult males, while consumption of wheat grain from plot B irrigated with CW pre-treated water is relatively safe.

The HI of the HMs to children, adult males and adult females were 2.45, 2.39 and 1.72, respectively for plot A while that for plot B were 0.86, 0.84 and 0.60, respectively the reduction rate of this risk reached 35.19% for different exposure populations. Pb made the highest contribution to integrative risk in both plot A and B. The HI of the HMs to different groups people were in the order of children>adult males>adult females. Through comparative study indicated that irrigation with CW

treated water can greatly reduce the HQ of individual HM and the accumulative risk of multiple HMs to people in this area.

CW reduced the risks by removing HMs in irrigation water and farmland soil efficiently. This suggested that CW could efficiently reduce the HMs in the contaminated YR water. The four HMs were reduced in a similar manner among four months, with June and July showing slightly lower reduction rate due to slow growth of cattail (producing less biomass) in these two months. The HMs reduction rate was not influenced by the concentration of HMs in YR water. The reduction rate of the four HMs went higher along with the time, with Cd being reduced mostly efficiently.

The concentrations of HMs in the CW substrate sludge were significantly increased with time. According to the BCF values, the HMs enrichment rates of cattail for HMs and that for different cattail tissues were Cd>Zn>Cu>Pb and root>leaf>stem, respectively. In this study, CW treated 1,350m<sup>3</sup> water every month with hydraulic retention time of 3 days.

Guerra et al. (2012) conducted a study to investigate the concentrations of the heavy metals Cd, Ni, Pb, Co and Cr in the most frequently consumed foodstuff (vegetables, fruits and cereal) in the São Paulo State, Brazil and compared with the permissible limits established by the Brazilian legislation and intake of heavy metals in human diets was also calculated to estimate the risk to human health using Hazard Quotient and Hazard Index.

All sampled vegetables presented average concentrations of Cd and Ni lower than the permissible limits established by the Brazilian legislation. Pb and Cr exceeded the limits in 44 % of the analyzed samples. Regarding the consumption habit of the population in the São Paulo State, the daily ingestion of heavy metals was below the oral dose of reference, therefore, consumption of these vegetables can be considered safe and without risk to human health.

The concentrations of Cd and Ni ranged between 0.01- 0.18 mg/kg and 0.01 - 0.74 mg/kg, and did not exceed the Brazilian limit for all vegetables, while the concentrations of Pb and Cr ranged between 0.02 - 2.50 mg/kg and 0.01 - 0.60 mg/kg, respectively and about 44.6 % of the samples exceeded the permissible limit for Pb and 44.2 % of Cr mainly for green vegetables accounting for 59.9 % of the total. The highest concentrations of Pb and Cr exceeded the permissible limits by approximately 5 (national garlic) and 6 (Japanese pumpkin) times, respectively. The fruits previously

described had Cd concentrations below the codex limit. Pb concentrations exceeded the codex limit, except for apples and 'hawai' pineapple.

The greatest contribution for daily intake for Cd came from the 'monalisa' potato (0.00049 mg per day), followed by the 'prata' banana (0.00046 mg per day), 'pêra' orange (0.00041 mg per day) and carrot (0.00032 mg per day), accounting for 22.6 % of the total daily intake. The greatest contribution for the Ni intake came from rice (0.015 mg per day) and bean 1 (0.0036 mg per day), accounting for 40.7 % of the total daily intake. Rice contributed with approximately 16.84 % for the Pb intake, followed by the 'pêra' orange (6.68 %), bean 2 (5.73 %), 'prata' banana (5.52 %) and onion (4.59 %). Rice was the major contributor for Co intake with 0.013 mg per day (33.98 %) followed by cassava with 0.0018 mg per day (4.54 %). The greatest contribution for Cr intake came from 'prata' banana (0.0014 mg per day), followed by 'nanica' banana (0.0013 mg per day) and carrot (0.0012 mg per day), accounting for 24.7 % of the total daily intake.

HQs for individual vegetables were all below 1.0 for all HMs, regardless the population type. HQ for Cd, Ni, Pb and Co ranged from 0 to 0.0071; from 0 to 0.0107; from 0 to 0.0701 and from 0 to 0.0045 for adults and from 0 to 0.0075; from 0 to 0.0114; from 0 to 0.0747 and from 0 to 0.0048 for children. The sequence of HQ for adults and children followed the decrescent order Pb>Cd>Ni>Co>Cr, however, the HQ for children was higher than that for adults. HI for adults and children were 0.57 and 0.68, respectively. The relative contributions of Cd, Ni, Pb and Co to the aggregated risk were 18.8 %, 5.7 %, 73.1 % and 2.3 % for adults and 19.1 %, 5.7 %, 73.4 % and 2.4 % for children, respectively.

Masona et al. (2011) conducted a study to determine the effects of long term wastewater irrigation on the concentrations of heavy metals (Zn, Cu, Mn, Cd, Pb, Ni, Fe and Cr) in soil, and their subsequent accumulation in maize plants at Firle Sewage Works in Harare, Zimbabwe having 30 years irrigation history with wastewater. The study revealed that long term wastewater use for irrigation results in heavy metal accumulation in soils and bioaccumulation in plants beyond maximum permissible limits (MPL) for both humans and livestock consumption. The order of Transfer factor between soil and maize plant was Pb (0.59) >Zn (0.42) >Mn (0.36) >Cu (0.05) >Fe (0.025), respectively. Thus, Lead had the highest and iron had the least transfer factor. The soil pH was found to be less acidic (pH = 5.6) in soils exposed to waste water than in soils where no wastewater had been applied (pH = 5).

Mishra and Tripathi (2008) conducted a study to determine the heavy metal contamination in soil with accumulation in plants in waste water irrigated areas. Results revealed that waste water contained lower concentrations of Cr, Zn, Cu, and Pb except Cd (0.03) than the permissible limits prescribed by the WHO. The concentrations of some metals were higher in vegetables and soils irrigated with treated waste water than those irrigated with ground water. Cd concentration was within the acceptable limits for food production in soil and vegetables at each site. However, Pb concentrations in vegetables exceeded levels considered as potentially dangerous (0.3 mg/kg for leafy vegetables). The maximum metal concentrations occurred in *Brassica oleracea* (Zn 63.80, Cu 12.25, Cr 10.65, Pb 3.63, and Cd 0.56 mg/Kg).

The metal enrichment (EF of Cd 1.9, Cr 2.9, Zn 4.8, Cu 6.5, and Pb 15.5) and degree of contamination (CF of Cd 2.9, Cr 2.0, Zn 2.3, Cu 2.7, and Pb 2.2) showed that accumulation of the five toxic metals increased during sewage irrigation as compared with the reference values. Based on WHO standards for heavy metal contamination of soil and irrigation water does not ensure safe levels for food.

Ghosh et al. (2012) undertaken a study in peri urban vegetable growing areas of Varanasi to investigate the long-term (15 years) impact of irrigation with treated sewage water (TSW) on Cd, Cr, Ni and Pb build-up in soils and its subsequent transfer into commonly grown vegetable crops and assessed the potential health risk associated with their consumption.

Results indicate that TSW was richer in essential plant nutrients but contained Cd, Cr and Ni in amounts well above the permissible limits for its use as irrigation water. Long-term application of TSW resulted in significant build-up of total and DTPA extractable Cd, Cr, Ni and Pb over GW irrigated sites. TSW also resulted in slight lowering in pH, increase in organic carbon (1.6 g/kg) and cation exchange capacity (5.2 cmol /kg).

Based on the tissue metal concentration and transfer factor value radish, turnip and spinach were grouped as hyper accumulator of heavy metals whereas brinjal and cauliflower accumulated less heavy metal. Health risk assessment by consumption of vegetables grown with TSW indicated that all the vegetables were safe for human consumption. However, significant accumulation of these heavy metals in soil and plant needs to be monitored.

The significant build-up of Cd (280%), Cr (312%), Ni (306%) and Pb (162%) was observed in soils using TSW for irrigation over those using groundwater. DTPA extractable Cd in the polluted sites ranged between (0.1-1.4 mg/kg), Ni (0.5-1.8 mg/kg), Pb (0.3-2.3 mg/kg) whereas no Cr was detectable in the DTPA extract.

Vegetables can be categorized into three groups on the basis of their cumulative uptake of Cd, Cr and Ni. Radish, cabbage and spinach had high accumulation of heavy metals; followed by intermediate accumulation by coriander, bean, tomato, turnip and carrot while potato, brinjal and cauliflower had the lowest accumulation of heavy metals in their edible parts. Vegetables where root is the edible part (radish and turnip) accumulated more Cd and Cr than where leaf is the edible part (spinach, coriander, cabbage), followed by plants where fruit/curd is the edible part (brinjal, cauliflower). Cabbage has been found to accumulate more Ni than other vegetables. However, the concentration of all the metals studied was found below the maximum permissible limit in vegetables.

The metal transfer factor varied from 0.013 in brinjal to 0.203 in spinach for Cd, 0.002 in brinjal to 0.038 in radish for Cr and 0.014 in brinjal to 0.116 in cabbage for Ni. Cd shown the highest metal transfer factor followed by Ni and Cr, suggesting that Cd is more bio-available. The transfer factor increased with increase in total metal content in soil, indicating a direct relationship between metal content in soil and plant. Genotypic differences in plant metal uptake has been observed in the present study with root crops being more efficient in accumulating heavy metals over leafy vegetables, followed by crops where fruit is edible part. Brinjal and cauliflower may be grouped as metal excluders whereas radish, turnip and spinach as metal accumulators. The Daily intake of metal (DIM) in the study area through consumption of contaminated vegetables was found to be far below the Reference dose (RfD) limit set by US-EPA. The Health Risk Index (HRI) for Cd from average consumption of vegetables was  $1.3E-01$  for adults and  $1.5E-01$  for children, for Cr  $6.1E-05$  for adult and  $7.0E-05$  for children and for Ni  $1.7E-02$  for adult and  $2.0E-02$  for children. Cd exhibited higher HRI followed by Ni and Cr but the HRI for all the metals studied was  $<1$ .

## MATERIAL AND METHODS

---

### 3.1 GENERAL SITE CHARACTERISTICS

The IARI campus is located in the heart of the NCT, Delhi (India) and is traversed by a network of sewage drains whose total discharge amounts to about 700-ham/ year (or about 20 MLD). These sewage drains receive domestic and industrial effluents generated by the IARI campus and a complex combination of industrial, commercial, agricultural and dwelling units around IARI micro-watershed (Figure 3.1). The sewage waters are associated with foul smell, dark black colour, high turbidity, high residual sodium carbonate (RSC), high bio-chemical oxygen demand (BOD) and appreciable lead, chromium, nickel and cobalt concentrations. General characteristics of the IARI micro-watershed are presented in (Table 3.1). About 290-ha of IARI micro-watershed comprises of farm area where agriculture is the major activity. Total annual irrigation demand of IARI farm has been estimated to be about 285-ham (or 7.8 MLD; Singh, 2004). About 163- ham (or 4.47 MLD) of this irrigation demand is met through groundwater and 20- ham (or 0.55 MLD) through canal water supplies. Thus IARI farm has an annual irrigation water deficit of about 101.5- ham (or 2.78 MLD). In absence of proper freshwater, in seed production unit farms (around the experimental field) raw & diluted sewage water is being used for irrigation. In order to circumvent the irrigation water deficit a pilot constructed wetland technology has been set up to re-use part available (700- ham) sewage waters, after some treatment, on the adjacent experimental micro-plots (Figure 3.2 and 3.3). The experimental micro-plots are located near a domestic sewage water drain on a 350 m<sup>2</sup> land area (28°38'21.3" N and 77°08'56.5" E) within the Seed Production Unit (SPU) farm of the Indian Agricultural Research Institute (IARI).

### 3.2 DESIGN AND OPERATION

#### 3.2.1 Experimental wastewater treatment system

The experimental wastewater treatment system is based on vertical sub-surface flow (VSSF) constructed wetland technology and comprises of 18-treatment cells (or mesocosms) assembled from commercially available 500-L capacity syntax

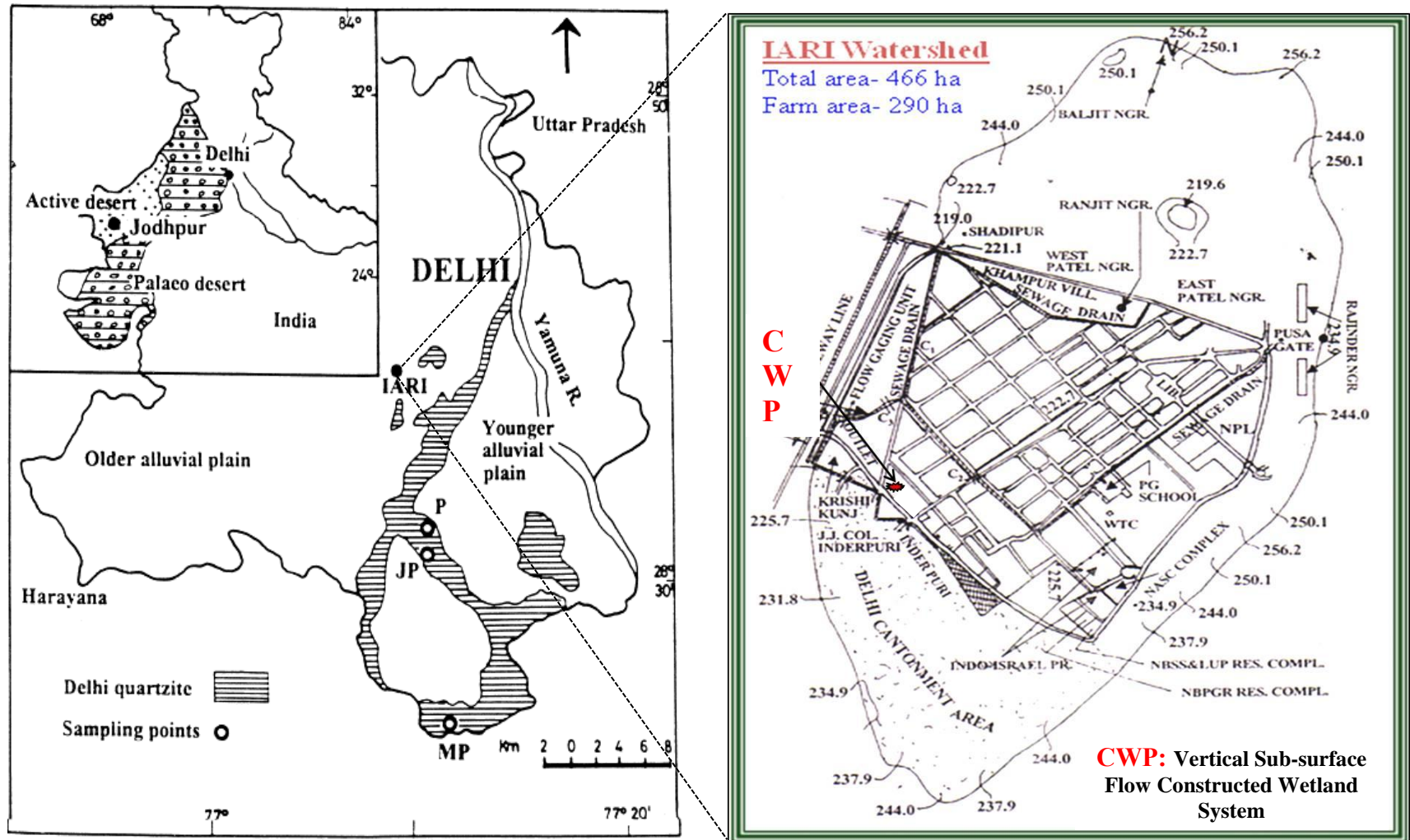


Figure 3.1: Location of project site within IARI micro-watershed

Table 3.1: General characteristics of IARI micro-watershed

<b>S. No.</b>	<b>Particulars</b>	<b>Values</b>
1.	Location a) Latitude b) Longitude	28.08°N 77.12°E
2.	Gross Area (ha) a) Area within IARI campus (ha) b) Area outside IARI campus (ha) c) Hilly Area	1031.24 466.06 474.43 90.75
3.	Land-use pattern a) Urban (ha) b) Agricultural & Horticultural (ha) I. Irrigated (ha) II. Un-irrigated (ha) c) Forest (ha)	650 290 275 15 90
4.	Elevation (Above mean sea level, in meters)	230
5.	Mean slope (%)	8.40
6.	Drainage Density (km/ km <sup>2</sup> )	0.93
7.	Topography	Rolling to Flat & Hilly
8.	Geology	Alluvium (Alwar quartzite of Delhi system)
9.	Climate a) Type b) Mean annual temperature (°C) c) Mean annual Rainfall (mm) d) Maximum rainfall receiving months	Sub-tropical 24 769 July to September
10.	Soil (Project site) a) Type b) Infiltration rate (mm/day) c) Field capacity (% g/g) d) Wilting point (% g/g) e) Bulk density (g/ cm <sup>3</sup> )	Sandy loam 117.0 23.87 10.71 1.54

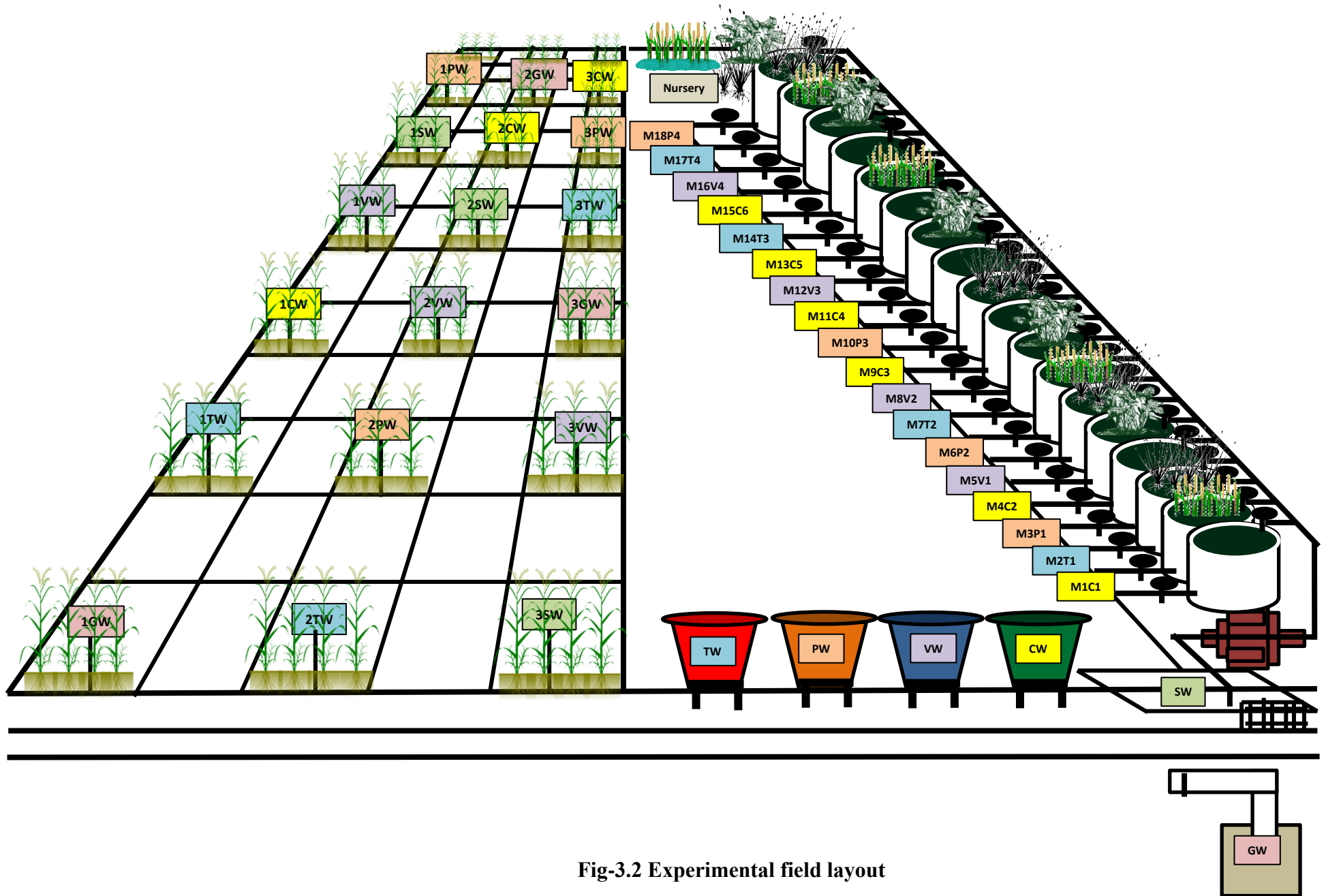


Fig-3.2 Experimental field layout



**Figures 3.3:** (a) Experimental wastewater treatment system based on vertical sub-surface flow (VSSF) constructed wetland technology (b) Experimental micro-plots

tanks. Each tank measures 0.89m (height) x 0.906 m (dia.) and has a surface area of approximately 0.645 m<sup>2</sup>. These are filled with a bottom layer (0.20m) of fine gravel (approximately 1.5 cm diameter and 38% porosity); middle layer (0.30 m) of coarse sand (approximately 0.2 cm diameter and 32% porosity) and a top most layer (0.20m) of native (sandy loam) soil (of 42% porosity, Figure 3.3) to obtain an effective pore space of 36.5% and an actual hydraulic retention time (HRT) of  $8.46 \pm 3.12$  hrs. All tanks were painted white, to avoid excessive heating of the outside surface or the inside media / micro-flora.

The mesocosms were connected to the individual inlet pipes, from the main (sewage water) influent discharge line, through ball valves to hydraulically maintain a maximum water level of 16.25cm (6.5 inches) above media, at each flooding event. To avoid any pump break-downs due to the choking of the inlet pipe, from floating plant/ plastic material, the untreated domestic wastewater in the open sewage channel is first passed through a perforated inlet gate and collected in a sump (2.36 x 0.68 x 0.762 m<sup>3</sup>) from where it is eventually pumped (through the main sewage water influent line) to the individual mesocosms. The wastewater flowing from open sewage channel to sump is gravity-driven while that from the sump to the individual mesocosms is pump – driven. The rest of the flow from the mesocosms to the main effluent line is again gravity driven. Each flooding event (for a set of 18-mesocosms) requires approximately 45 minutes–pump (2 HP) operation.

Four replicates of three emergent macrophytes viz. *Typha latifolia* (Cattail, T1), *Phragmites karka* (Reed, T2), *Acorus calamus* (Vechh, T3), were planted in 12-mesocosms while the remaining 6-mesocosms were left un-vegetated (T4) and thus represented a control treatment. In the vegetated mesocosms, plants were allowed to grow and multiply to form a dense stand in the mesocosms with periodic application of wastewater as a source of nutrients for the plants, before the actual start for the experiment from November, 2010 onwards. Dead shoots were replaced with the new ones randomly until the growth stabilized in the mesocosm media.

The mesocosms were intermittently flooded (to a maximum depth of 6.5 inches) during the first experimental period (Nov. 2010 to March 2011), thrice in a month after every 20 days interval and during the second experimental period (June 2010 to November 2010), at every alternate day. The treated effluent waters from each mesocosm were collected in 18 liter capacity pre-labelled white plastic buckets

and the similar treatment effluents were mixed in 110 liter capacity pre-labelled big buckets to get a homogenous treatment effluent mixture. The effluent water mixture were sampled and then used for irrigating the adjacent experimental micro-plot crops. On the day of field irrigation, the mesocosms were completely drained and re-flooded. Thus in general the aforementioned mesocosm-flooding events coincided with the field-irrigation days during *Rabi* (Nov. – March) and *Kharif* (June – Nov.) crop growing seasons.

### 3.2.2 Micro-plot experimental field

The experimental field was located adjacent to the experimental treatment plant (mesocosms) consisting of 18 micro-plots, each of size 1.8m × 1.5m (= 2.7 m<sup>2</sup>). The field had pre-contaminated history with sewage and having sandy loam type soil. The six treatments viz. *Typha latifolia* (Cattail) treated water (TW), *Phragmites karka* (Reed) treated water (PW), *Acorus calamus* (Vacha) treated water (VW), Control (No vegetation) treated water (CW), Raw Sewage water (SW) and Ground water (GW) irrigated micro-plots were arranged in randomized block design (RBD), with three replications.

The experimental field was properly ploughed, planked and levelled. Micro-plots were prepared, lined by bund and tagged during Nov. 2010 for Wheat and puddled during June 2011 for Paddy. A basal fertilizer dose (25kg Urea/ acre, 50kg DAP/acre and 25kg MOP/acre) was applied manually as 35g Urea/plot, 17.5g DAP/plot and 17.5g MOP/plot in both Wheat and Paddy.

Wheat (Variety HD-2894) seeds were sown on 26<sup>th</sup> Nov. 2010 at 2-3 cm depth with 22.5 cm row to row and 10 cm plant to plant spacing having 6 rows in each micro-plot. While in case of paddy 23 days old Paddy (Variety Pusa Basmati-1121) seedlings were procured from Seed Production Unit (IARI) nursery and transplanted on 8<sup>th</sup> July 2011 at 4cm depth with 3 seedlings per hill and 20 cm row to row and 10 cm plant to plant spacing in each microplot. Thinning & gap filling was done to maintain almost uniform plant population and proper spacing.

Three irrigations per month with mesocosm - effluents, raw sewage water and groundwater, at approximately 20 days interval, were applied in the corresponding micro-plots during wheat crop season (Nov. 2010 – March 2011) and on every alternate day during paddy crop season (June 2011 - Nov. 2011). During entire experimental period, every micro-plot received about 54 litres of irrigation water on each irrigation day.

Topdressing of urea was done on 27<sup>th</sup> Dec. 2010 in wheat crop during crown root initiation stage @ 8g/plot in each micro-plot while in paddy crop topdressing of urea was done on 12<sup>th</sup> Aug. 2011 and 15<sup>th</sup> Sept. 2011 during tillering and panicle initiation stage respectively.

Regularly hand weeding was done inside and adjacent to the micro-plots. Termite attack was prominent here, so to control them chlorpyrifos (50% EC) was used to prepare a chemical solution (@ 5ml/litre in water) and 80 ml of it was applied to each micro-plot by dissolving into the last bucket of 16 litres of irrigation water on 17<sup>th</sup> March 2011, in wheat crop and on 10<sup>th</sup> Sept. 2011, in paddy crop. On the sign of attack of fungus, paddy blast, a fungicide Tilt 250 EC was sprayed by dissolving 1ml/litre in water on (23<sup>rd</sup> Sept. 2011) and for stem borer infection Caldon 50 SP 0.1% was applied @ 1.5 handful granules/ plot on (3<sup>rd</sup> Oct. 2011) in paddy crop.

After grain filling stage in wheat season twelve healthy plants (four each of Long, medium and short height) and in paddy season three tillers of five healthy plants (long, medium and short height) were tagged and number of termite & fungus infected plants/tillers were counted along with the total plants per micro-plot. About 2 weeks before harvesting of the crops, irrigation was stopped. Intact rooted tagged plants were harvested first by khurpi and transferred to the laboratory. While the rest crop plants of corresponding micro-plot were harvested by sickle individually (15<sup>th</sup> April 2011 for wheat & 14<sup>th</sup> Nov. 2011 for Paddy), tied in bundles by stripes and transferred to the laboratory. Crop roots were also uprooted from the micro-plot individually and after placing in polythene sheet transferred to laboratory. Different micro-plot harvested crop plants along with their roots were air dried and weighed by balance individually. Thereafter threshing was done of individual micro-plot harvested crop plants and their grains were weighed by weighing balance and then after filling in cloth bags transferred to the laboratory.

On the tagged plants, different plant parameters were analysed and the separated root, shoot & grain samples of each micro-plot were packed into separate brown paper envelopes. Root & shoot samples were oven dried. Grains, oven dried root & shoot samples were ground in milling machine separately and subjected to metal analysis. Test weight (both 100 & 1000 seed weight) of the harvested grains of each micro-plot grains was taken by seed counter individually, for three replications. About 350 harvested grains of each micro-plot were taken for seed germination test in

seed testing laboratory of Seed Science Technology Division (IARI), in three replicates.

### **3.3 SOIL, WATER, PLANT SAMPLING AND ANALYSIS**

#### **3.3.1 Sampling Plan**

In order to assess the impact of irrigation water on the soil, plant & agricultural produce; the soil, water and plant samples from each treatment were periodically collected.

##### *Water sampling*

The water sampling dates coincided with the field-irrigation/ the mesocosm-flooding dates. The effluent water samples from each mesocosm, sewage water & groundwater samples, which were used for irrigation to the adjacent micro-plots, were collected thrice every month at approximately 20 days interval during Rabi (Nov. – March) and on alternate day during Kharif (June – Oct.). In Kharif season, every month a composite irrigation sample of 300ml was prepared. The pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), oxidation-reduction potential (ORP), salinity and temperature of the water samples were determined *in-situ*. While for the remaining chemical analysis (Table 3.2), the water samples were immediately transferred from the site to the laboratory where they were stored in a refrigerator at 4°C until further processing/ analysis, as per the standard procedures illustrated in (Table 3.2) and details in (section 3.3). Each water sample was collected in two (500 ml and 100 ml capacity) high density polyethylene bottles. The water samples collected in the 100 ml capacity plastic bottles were immediately acidified with 1 N HNO<sub>3</sub> and subjected to heavy metal analysis while the water samples collected in the 500 ml capacity plastic bottle were subjected to the remaining physico-chemical tests (Table 3.2). Prior to sample collection, the plastic bottles were rinsed with respective waters, for at least two to three times and were closed air tight, after collection, to avoid any sample contamination/ loss due to spillage.

Table 3.2: List of physico-chemical tests performed on the water samples

<b>Parameter(s)</b>	<b>Method(s)</b>	<b>Reference(s)</b>
pH	Multi- parameter analyser	
EC		
TDS		
ORP		
Temperature		
Salinity		
Turbidity	Nephelometer	Jackson (1973)
Carbonate & Bi- carbonate Concentration	Titration method	Richards (1954)
Calcium & Magnesium Concentration	Versenate method	Jackson (1973)
Sodium Concentration	Flame photometer	Jackson (1973)
Potassium Concentration	Flame photometer	Jackson (1973)
Chloride Concentration	Argentometric method	Clesceri et al. (1998)
Fluoride Concentration	SPADNS method	Bellack & Schouboe (1968)
Sulphate Concentration	Turbidimetric method	Clesceri et al. (1998)
Nitrate Concentration	UV- spectrophotometry	Clesceri et al. (1998)
Phosphate Concentration	Amino acid method	Clesceri et al. (1998)
Heavy Metal (viz. Zn, Mn, Fe) Concentrations	Atomic Absorption Spectrophotometer	Willis (1962)
Heavy Metal (viz. Cu, Cd, Cr, Co, Pb) Concentrations	Polarography	Wang (1989)

### *Soil sampling*

Besides, soil samples from each experimental plot were collected both before and during each cropping season. In case of wheat, one soil sampling was done just before sowing (Nov. 2010), one at mid-crop season (Feb. 2011) and one at the end of the cropping season (April 2011). While in case of paddy as the last soil sample for wheat season was assumed as the initial sample for paddy season therefore, only one soil sampling was done at the end of the paddy cropping season (Nov. 2011). The soil samples of the top (0-30 cm) layer from each micro-plot were collected randomly from five spots by core sampler to form a bulk (500 g) soil sample. These soil samples were packed in pre-marked polyethylene bags and immediately transferred to the laboratory where these were processed (i.e. air dried, crushed, ground and sieved through a 2-mm sieve) and subjected to a number of physico-chemical tests (as illustrated in Table 3.3), as per the procedures detailed in section (3.3.2.2).

### *Plant sampling*

For analysing the impact of water treatments on crop plant and produce, plant samples were also collected. In case of wheat, four plants each of three height groups viz. long (Main), medium (Mid) & short (Low) of total twelve healthy plants and in case of paddy, three tillers of three height groups viz. long (Main), medium (Mid) & short (Lower) of total five healthy plants per each micro-plot were randomly tagged, just after grain filling stage, and used for analysis. These tagged plants having intact root were sampled with khurpi just before harvesting of the rest of the plants. Each micro-plot plant sample was bundled and immediately transferred to laboratory and air dried. On the air dried plant samples different plant parameters (given in section 3.3.2.3, and Table 3.4) were analysed and the separated root, shoot & grain parts were packed in brown envelopes. Root & shoot parts were oven dried (at 55°C) and ground (individually) along with grains through a milling machine and subjected to micro-nutrient (viz. Zn, Cu, Fe, Mn) and trace metal (viz. Cd, Cr, Co, Ni, Pb) analysis as per the procedure detailed in section (3.3.2.3).

All samples were analysed in triplicate; with due quality control ensured through careful standardization, procedural blank measurements and duplicate samples in the Hydro-informatics and Wetland bio-geochemistry laboratories of the Division of Environmental Sciences, IARI.

Table 3.3: List of physico-chemical test performed on soil samples

<b>Parameter(s)</b>	<b>Method(s)</b>	<b>Reference(s)</b>
pH	Soil : Water extract (1:5)	Jackson (1973)
EC	Soil : Water extract (1:5)	Jackson (1973)
Oxidisable Organic Carbon	Chromic acid oxidation method	Walkley and Black (1934)
Carbonate & Bi- carbonate Concentrations	Titration method	Richards (1954)
Calcium & Magnesium Concentrations	Versenate method	Jackson (1973)
Sodium Concentrations	Flame photometer	Jackson (1973)
Potassium Concentrations	Flame photometer	Jackson (1973)
Chloride Concentrations	Argentometric method	Clesceri et al. (1998)
Fluoride Concentrations	SPADNS method	Bellack & Schouboe (1968)
Sulphate Concentrations	Turbidimetric method	Clesceri et al. (1998)
Nitrate Concentrations	UV- spectrophotometry	Clesceri et al. (1998)
Phosphate Concentrations	Olsen's method	Olsen et al. (1954)
Heavy Metal (viz. Zn, Fe, Mn) Concentrations (Bio-available + Total)	Atomic Absorption Spectrophotometer	Willis (1962)
Heavy Metal (viz. Cu, Cd, Cr, Co, Pb) Concentrations (Bioavailable + Total)	Polarography	Wang (1989)

Table 3.4: List of parameters performed on plant samples

<b>Parameter</b>	<b>Method</b>	<b>Reference</b>
Shoot length Root length Spike/Panicle length Shoot weight Root weight Spike/ Panicle weight Grain number per Spike/Panicle Grain weight per Spike/Panicle Unfilled seed per Panicle Infected plant/Tillers per microplot Economic Yield (per micro-plot) Biological Yield (per micro-plot)	Manual (by scale & weighing balance)	
Seed Germination	Rolled towel method	ISTA rules (Anon.,1985)
Heavy Metal (viz. Zn, Mn, Fe) Concentrations	Atomic Absorption Spectrophotometer	Willis (1962)
Heavy Metal (viz. Cu, Cd, Cr, Co, Pb) Concentrations	Polarography	Wang (1989)

### 3.3.2 Sample analysis

#### 3.3.2.1 Water analysis

*Electrical Conductivity, pH, Total Dissolved Solids, Oxidation Reduction Potential, Temperature, Salinity*

The pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), oxidation-reduction potential (ORP), salinity and temperature of the influent and effluent water samples were determined in-situ by means of a HORIBA U-51 probe. A standard solution of pH- 4 and EC- 4.49 dS/m was used for the calibration of the probe. Thereafter the probe (housed in a sensor guard) was dipped in the influent/effluent water samples and the measurements were recorded/ saved in the equipment memory.

*Turbidity*

Turbidity of influent/effluent waters was analyzed by means of a turbidimeter (Hach-2100P-Turbidimeter). For this, the influent/effluent water samples were filled in a sample cell (glass vials) and the turbidity was directly read from the instrument's display. Formazin standard solutions of turbidity <0.1 NTU, 20 NTU, 100 NTU and 800 NTU were used for the calibration of the Turbidimeter.

*Carbonate, Bicarbonate and Chloride Concentrations*

Carbonate ( $\text{CO}_3^{2-}$ ) bicarbonate ( $\text{HCO}_3^-$ ) and chloride ( $\text{Cl}^-$ ) concentrations (meq/l) of influent/effluent water samples were determined titrimetrically (Richards, 1954). For this 5-ml of the water sample and 20 ml of distilled water were taken in 150 ml conical flask. To this 1-drop of phenolphthalein indicator was added. On appearance of pink colour, titration was carried out against 0.01N sulphuric acid ( $\text{H}_2\text{SO}_4$ ) and volume of acid consumed was recorded to determine the carbonate concentration in the test water samples. Thereafter, two drops of methyl orange indicator were added to the colourless content in the flask (left after titration or fresh sample which failed to develop colour even after the addition of phenolphthalein indicator, due to the absence of carbonate) and titrated against 0.01N  $\text{H}_2\text{SO}_4$  for determining  $\text{HCO}_3^-$  concentrations in the test water samples. To the resultant sample solution, 1-mg of  $\text{CaCO}_3$  and 2 drops of potassium chromate indicator were added and titrated against standard 0.025N silver nitrate ( $\text{AgNO}_3$ ) solution for determining chloride

concentrations. Finally, carbonate, bicarbonate and chloride concentrations were calculated as per the following eq. (1, 2, and 3):

$$\text{CO}_3^{2-} \text{ (meq/l)} = (2 \cdot V_x \cdot N_a \cdot 1000) / V_s \dots (1)$$

$$\text{HCO}_3^- \text{ (meq/l)} = \{(V_x - V_y) \cdot N_a \cdot 1000\} / V_s \dots (2)$$

$$\text{Cl}^- \text{ (meq/l)} = \{(V_a - V_b) \cdot N \cdot 1000\} / V_s \dots (3)$$

where,  $V_x$  is the volume of 0.01N  $\text{H}_2\text{SO}_4$  consumed for complete neutralization of carbonates,  $V_y$  is the volume of 0.01N  $\text{H}_2\text{SO}_4$  consumed for complete neutralization of bicarbonates,  $N_a$  is the normality of the acid used,  $V_a$  is the volume of 0.025N  $\text{AgNO}_3$  consumed by the water sample,  $V_b$  is the volume of 0.025N  $\text{AgNO}_3$  consumed by the blank (double distilled water),  $N$  is the normality of the  $\text{AgNO}_3$  used and  $V_s$  is volume of water sample taken for analysis.

#### *Calcium and Magnesium Concentrations*

Calcium (Ca) and Magnesium (Mg) concentrations (meq/L) of influent/effluent water samples were determined by Versenate (Ethylene-Di-Amine-Tetra-Acetic Acid, EDTA) titration method (Jackson, 1973). For this, 5 ml of water sample was taken in 100 ml conical flask and to it 20 ml of distilled water was added. This was followed by the addition of 1 ml of ammonium hydroxide - ammonium chloride buffer solution (of pH 10.0) and 3-4 drops of Erichrome Black T indicator and titrated against 0.01N EDTA to obtain calcium and magnesium concentrations as per the following equation (4):

$$\text{Ca}^{2+} + \text{Mg}^{2+} \text{ (meq/L)} = (V_a \times N_a) / V_s \times 1000 \dots (4)$$

where,  $V_a$  is volume of EDTA used in titration,  $V_s$  is volume of water sample taken and  $N_a$  is normality of EDTA.

#### *Sodium Concentrations*

Sodium concentrations (Na, ppm) in the influent/ effluent water samples were determined by means of a flame photometer (Elico CL-361, Jackson, 1973). For this a stock sodium solution (100 ppm Na-concentration) was prepared by dissolving 0.2587 g of AR grade NaCl in 1L-distilled water. Standard sodium solutions of 0, 5, 10, 20, 40, 60 and 80 ppm were prepared from the stock solution to prepare the calibration

curve. Thereafter the soil extracts were introduced into the pre-calibrated flame photometer to obtain their sodium concentrations (ppm).

#### *Potassium Concentrations*

Potassium concentration (K, ppm) of influent/effluent water samples were also determined through a flame photometer (Jackson, 1973). For this a stock sodium solution (100 ppm K-concentration) was prepared by dissolving 0.1907 g of AR grade KCl in 1L-distilled water. Standard sodium solutions of 0, 5, 10, 20, 40 and 60 ppm were prepared from the stock solution to prepare the calibration curve. Thereafter the soil extracts were introduced into the pre-calibrated flame photometer to obtain their potassium concentrations (ppm).

#### *Fluoride Concentrations*

Fluoride concentrations (F, ppm) of influent/effluent water samples were estimated following SPADNS colorimetric method (Bellack & Schouboe, 1968) which is based on the reaction between fluoride and Zirconium Alizarin Lake. Fluoride reacts with the alizarin dye dissociating a portion of it into a colourless complex anion ( $ZrF_6^{2-}$ ) and the dye. Thus, lighter the colour of the complex more is the fluoride in the medium. The colour developed in the sample was read by means of a UV-VIS spectrophotometer at 580 nm wavelength and the fluoride concentrations were estimated by means of a standard curve using 0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 1.2 and 1.4 ppm F standard solutions.

#### *Nitrate Concentrations*

Nitrate concentrations ( $NO_3^-$ , ppm) of influent/effluent water samples were determined by ultraviolet spectroscopic screening method (Clesceri *et al.*, 1998). Absorbance of samples was read at 220 nm. A second measurement at 275 nm (associated with zero absorbance due to any nitrates) was also made to correct for a change in  $NO_3^-$  concentrations due to the presence of dissolved organic matter (with absorbance at both 220 nm and 275 nm). The nitrate concentrations were estimated by means of a standard curve using 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ppm  $NO_3^-$  standard solutions. The above standards were made from a stock solution of 100 ppm  $NO_3^-$  (prepared by dissolving 0.7218 g of AR grade  $KNO_3$  in 1 L-distilled water). 0.5 ml of 1N HCl was

also added to 5 ml water sample to prevent interference from hydroxide or carbonate ions.

#### *Sulphate Concentrations*

Sulphate concentrations ( $\text{SO}_4$ , ppm) were estimated by turbidimetric method (Clesceri *et al.*, 1998). For this, 10ml of water sample was taken in a 25ml volumetric flask. Then 2ml of buffer solution and a pinch of  $\text{BaCl}_2$  crystals were added to it. The mixture was stirred for 60 seconds and poured into an absorbance cell to measure its turbidity after 5 minute at 420nm wavelength in a UV-VIS spectrophotometer. Blank was run without adding  $\text{BaCl}_2$ . Buffer solution was prepared by dissolving 30 mg  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 5g Sodium Acetate, 1g Potassium nitrate and 20 ml Acetic acid in 1000 ml distilled water. A standard stock solution of 1000 ppm was made by dissolving 1.479 g anhydrous  $\text{Na}_2\text{SO}_4$  in 1000 ml distilled water. Calibration curve was prepared by spacing standards at 5 ppm increments in the 0 – 40 ppm  $\text{SO}_4^{2-}$  range i.e. 0, 5, 10, 15, 20, 25, 30, 35 and 40 ppm. Corrections were made for each water sample by running blanks to which  $\text{BaCl}_2$  was not added.

#### *Phosphate Concentrations*

Phosphate concentrations ( $\text{PO}_4$ , ppm) were determined through an amino acid method by means of a UV-VIS spectrophotometer (Hach DR-5000). For this 5-ml of water sample was mixed with 0.2 ml of amino acid reagent (HACH reagent) and 0.2 ml of molybdate reagent (HACH reagent). In a highly acidic solution, ammonium molybdate reacts with orthophosphate to form molybdophosphoric acid. This complex is then reduced by the amino acid reagent to yield an intensely coloured molybdenum blue compound. The mixture was incubated for 10 minutes. Thereafter its absorbance was taken at 530nm. Calibration curve was prepared by using  $\text{PO}_4^{3-}$  standards at 5, 8, 10, 12, 15, 18, 20 and 25 ppm from a 1000 ppm stock solution prepared by dissolving 438 mg anhydrous  $\text{KH}_2\text{PO}_4$  in 1000ml distilled water.

#### *Heavy Metal Concentrations*

To determine heavy metal (viz., Cu, Fe, Mn, Zn, Cr, Ni, Cd, Co, Pb) concentrations in the influent/effluent water samples, acidified water samples were digested by using di-acid mixture ( $\text{HNO}_3:\text{HClO}_4 :: 9:4$ ). For this 100ml of sample was taken in a 250 ml conical flask, to this 15 ml of di-acid mixture was added and placed on a hot plate

maintained at a temperature of 70°C. Digested samples were filtered in 100 ml volumetric flask and 100 ml volume was made up by using deionised water. After filtration, concentrations of Fe, Mn and Zn were determined through an atomic absorption spectrometer (ECIL AAS4141 model) while those of Cu, Ni, Cd, Cr, Co, and Pb were determined by means of a Polarographic Trace Lab Analyzer (POL150 model; Wang, 1989).

### 3.3.2.2 Soil analysis

#### *Electrical Conductivity*

Electrical conductivity (EC) of 1:5 (Soil: Distilled water) extracts in 20 ml beakers were determined by means of a pre-calibrated conductivity meter (Jackson, 1973). A standard 0.01 M KCL solution (i.e. 0.7466 g of dry AR grade KCL in 1L distilled water) with 1.413- dS/m electrical conductivity at 25°C temperature was used for the calibration of the conductivity meter.

#### *pH*

The pH of 1:5 (Soil: Distilled water) extracts in 20 ml beakers were also determined by means of a pre-calibrated pH meter (Jackson, 1973). Buffer solutions of pH 7.0, 4.0 and 9.2 were used for the calibration of the pH meter.

#### *Oxidisable Organic Carbon*

Oxidisable organic carbon (OOC) was analysed through Chromic acid oxidation method (Walkley and Black, 1934). For this, 2g of air-dried soil was weighed in a 500 ml conical flask. Then 10 ml of 1N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution and 20 ml of conc. H<sub>2</sub>SO<sub>4</sub> were added to it. The mixture was kept in dark for 2 hours to cool the contents and to make the reaction complete. This was followed by addition of 100 ml of distilled water and 10 ml of ortho-phosphoric acid (88%) and the excess potassium dichromate was back titrated with 0.5 N ferrous ammonium sulphate in the presence of diphenyl amine indicator. This indicator gave violet colour to the solution. The end point was change of colour from violet to bright green. Finally the following formula (5) was used for the calculation of organic carbon:

$$\text{OOC (\%)} = [(x-y)/2] \times 0.003 \times (100/S) \times (100/76) \dots(5)$$

where, x is volume (ml) of 0.5 N ferrous ammonium sulphate used for blank titration, y is volume (ml) of 0.5 N ferrous ammonium sulphate used for sample titration and S is the amount of soil sample (g) taken for analysis.

#### *Sulphate Concentrations*

Sulphate concentrations ( $\text{SO}_4$ , ppm) of 1:5 (Soil: 500 ppm Monocalcium phosphate) extracts were determined by Turbidimetric method (Chesnin and Yien, 1950). For this, 10 ml of soil extract was taken in 25 ml volumetric flask. Then 1 g of 30-60 mesh  $\text{BaCl}_2$  crystals and 1 ml of 0.25% solution of gum acacia in distilled water were added to it and their volumes were made by adding distilled water. The turbidity developed in the sample was read by a UV-VIS spectrophotometer at 420 nm wavelength and the sulphate concentrations were estimated by means of a standard curve using 0, 1, 2, 3, 4, 5 and 6 ppm  $\text{SO}_4^{2-}$  standard solutions.

#### *Phosphate Concentrations*

Phosphate concentrations ( $\text{PO}_4$ , ppm) of 1:20 (Soil: 0.05N  $\text{NaHCO}_3$ ) extracts were determined by Olsen's method (Olsen et al., 1954). For this, 5 ml of soil extract was taken in 25 ml volumetric flask and 0.5 ml of 5N  $\text{H}_2\text{SO}_4$  and 4-ml of ascorbic acid solution were added to it and their volumes were made up to 25 ml by adding distilled water. The ascorbic acid solution was prepared by dissolving 1.056 g ascorbic acid salt in 200 ml of solution made from ammonium molybdate (12 g) and antimony potassium tartrate (0.2908 g). The blue colour developed in the sample was read by a UV-VIS spectrophotometer at 760 nm wave length and the phosphate concentrations were estimated by means of a standard curve using 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ppm  $\text{PO}_4^{3-}$  standard solutions made from a 1000 ppm stock solution, prepared by dissolving 438 mg anhydrous  $\text{KH}_2\text{PO}_4$  in 1000 ml distilled water.

#### *Carbonate, Bicarbonate and Chloride Concentrations*

Carbonate ( $\text{CO}_3^{2-}$ ) bicarbonate ( $\text{HCO}_3^-$ ) and chloride ( $\text{Cl}^-$ ) concentrations (meq/l) were determined titrimetrically (Richards, 1954). For this 5-ml of 1:5 (Soil: Distilled water) extracts and 20 ml of distilled water were taken in 150 ml conical flask. Then 1-drop of phenolphthalein indicator was added to it. On appearance of pink colour, titration was carried out against 0.01N sulphuric acid ( $\text{H}_2\text{SO}_4$ ) till become colourless and the volume of acid consumed was recorded to determine the carbonate concentration.

Thereafter, two drops of methyl orange indicator were added to the colourless content in the flask and titrated against 0.01N H<sub>2</sub>SO<sub>4</sub>. The consumed acid volume to change colour from orange to red during titration was recorded to determine the bicarbonate concentration. To the resultant sample solution, 1-mg of CaCO<sub>3</sub> and 2 drops of potassium chromate indicator were added and then titrated against standard 0.025N silver nitrate (AgNO<sub>3</sub>) solution till break red colour develop and consumed titrant volume were noted for determining the chloride concentrations. Finally, carbonate, bicarbonate and chloride concentrations were calculated as per the following eq. (6, 7, and 8):

$$\text{CO}_3^{2-} \text{ (meq/l)} = (2 \cdot V_x \cdot N_a \cdot 1000) / V_s \dots (6)$$

$$\text{HCO}_3^- \text{ (meq/l)} = \{(V_x - V_y) \cdot N_a \cdot 1000\} / V_s \dots (7)$$

$$\text{Cl}^- \text{ (meq/l)} = \{(V_a - V_b) \cdot N \cdot 1000\} / V_s \dots (8)$$

where, V<sub>x</sub> is the volume of 0.01N H<sub>2</sub>SO<sub>4</sub> consumed for complete neutralization of carbonates, V<sub>y</sub> is the volume of 0.01N H<sub>2</sub>SO<sub>4</sub> consumed for complete neutralization of bicarbonates, N<sub>a</sub> is the normality of the acid used, V<sub>a</sub> is the volume of 0.025N AgNO<sub>3</sub> consumed by the water sample, V<sub>b</sub> is the volume of 0.025N AgNO<sub>3</sub> consumed by the blank (double distilled water), N is the normality of the AgNO<sub>3</sub> used and V<sub>s</sub> is volume of water sample taken for analysis.

#### *Calcium and Magnesium Concentrations*

Calcium (Ca) and Magnesium (Mg) concentrations (meq/L) were determined by Versenate (Ethylene-Di-Amine-Tetra-Acetic Acid, EDTA) titration method (Jackson, 1973). For this, 5 ml of 1:5 (Soil: Distilled water) extract was taken in 150 ml conical flask and 20 ml of distilled water was added to it. This was followed by the addition of 1 ml of ammonium hydroxide - ammonium chloride buffer solution (of pH 10.0) and 3-4 drops of Erichrome Black T indicator and titrated against 0.01N EDTA and the titre volume for the colour change from wine red to sky blue was noted to obtain calcium and magnesium concentrations as per the following equation (9):

$$\text{Ca}^{2+} + \text{Mg}^{2+} \text{ (meq/L)} = (V_a \times N_a) / V_s \times 1000 \quad \dots (9)$$

where, V<sub>a</sub> is volume of EDTA used in titration, V<sub>s</sub> is volume of water sample taken and N<sub>a</sub> is normality of EDTA.

#### *Sodium Concentrations*

Sodium concentrations (Na, ppm) were determined by means of a flame photometer (Elico CL-361, Jackson, 1973). For this a stock sodium solution (100 ppm Na-concentration) was prepared by dissolving 0.2587 g of AR grade NaCl in 1L-distilled water. Standard sodium solutions of 0, 5, 10, 20, 40, 60 and 80 ppm were prepared from the stock solution to prepare the calibration curve. Thereafter the 1:5 (soil: ammonium acetate extractant) extracts were introduced into the pre-calibrated flame photometer to obtain their sodium concentrations (ppm).

#### *Potassium Concentrations*

Potassium concentration (K, ppm) were also determined through a flame photometer (Jackson, 1973). For this a stock sodium solution (100 ppm K-concentration) was prepared by dissolving 0.1907 g of AR grade KCl in 1L-distilled water. Standard sodium solutions of 0, 5, 10, 20, 40 and 60 ppm were prepared from the stock solution to prepare the calibration curve. Thereafter the 1:5 (soil: ammonium acetate extractant) extracts were introduced into the pre-calibrated flame photometer to obtain their potassium concentrations (ppm).

#### *Fluoride Concentrations*

Fluoride concentrations (F, ppm) were estimated by following the SPADNS colorimetric method (Clesceri et al., 1996) which is based on the reaction between fluoride and Zirconium Alizarin Lake. Fluoride reacts with the alizarin dye dissociating a portion of it into a colourless complex anion ( $ZrF_6^{2-}$ ) and the dye. Thus, lighter the colour of the complex more is the fluoride in the medium. The colour developed in the sample was read by means of a UV-VIS spectrophotometer at 580 nm wavelength and the fluoride concentrations were estimated by means of a standard curve using 0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 1.2 and 1.4 ppm F standard solutions.

#### *Nitrate Concentrations*

Nitrate concentrations ( $NO_3^-$ , ppm) were determined by ultraviolet spectroscopic screening method (Clesceri *et al.*, 1998). Absorbance of samples was read at 220 nm. A second measurement at 275 nm (associated with zero absorbance due to any nitrates) was also made to correct for a change in  $NO_3^-$  concentrations due to the presence of dissolved organic matter (with absorbance at both 220 nm and 275 nm).

The nitrate concentrations were estimated by means of a standard curve using 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ppm  $\text{NO}_3^-$  standard solutions. The above standards were made from a stock solution of 100 ppm  $\text{NO}_3^-$  (prepared by dissolving 0.7218 g of AR grade  $\text{KNO}_3$  in 1 L-distilled water). 0.5 ml of 1N HCl was also added to 5 ml water sample to prevent interference from hydroxide or carbonate ions.

#### *Heavy Metal Concentrations*

Soil samples were subjected to both bio-available and total metal analysis. To determine bio-available (i.e. DTPA extractable) soil heavy metal concentrations (viz. Zn, Cu, Cd, Cr, Co, Mn, Fe, Pb), 40 g of air dried soil was taken in 150 ml conical flask. To this 80 ml of DTPA solution was added and the contents were shaken for 2 hrs. and finally filtered. The DTPA extractant (Lindsay and Norvell, 1978) was prepared by dissolving 1.967 g of DTPA (di-ethylene-tri-amine-penta-acetic acid), 1.47 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and 13.3 ml of tri-ethanolamine (TEA) in 900 ml of distilled water. This was followed by adjusting the pH of DTPA solution to  $7.3 \pm 0.5$  with 1:1 HCl and making up the volume to 1 L.

While to determine total heavy metal concentrations, 0.5 g of soil sample was taken in a 150 ml conical flask and to it 15 ml of di-acid (i.e.  $\text{HNO}_3:\text{HClO}_4::9:4$ ) was added. The mixture was placed on a hot plate maintained at  $70^\circ\text{C}$  temperature and left for digestion. The digested samples were filtered in 100 ml volumetric flasks and their volume made up to 100 ml by means of the deionised water.

After filtration, the concentrations of Fe, Mn and Zn in both DTPA extractable and acid digested samples were determined through an atomic absorption spectrometer (ECIL AAS4141 model) while those of Ni, Cd, Cr, Co, Cu and Pb were determined by means of a Polarographic Trace Lab Analyzer (POL150 model; Wang, 1989).

#### **3.3.2.3 Plant analysis**

##### *Plant parameters analysis*

Harvested tagged plant samples were taken to lab and air dried. Root length, shoot length, spikelet length (wheat) and/or panicle length (paddy) were taken by manual scale and root weight, shoot weight, spikelet and/or panicle weight & grain weight were taken by weighing balance. Numbers of grains per spikelet and/or panicle were counted. In paddy number of productive, unproductive, infected and total tillers, per plant and numbers of unfilled kernels per panicle were also counted.

*Economic Yield, Biological Yield & Harvest index*

Bundles of separately harvested crop of each microplot and their roots were air dried and weighed by weighing balance. The threshed grains of each microplot were also weighed by weighing balance. These were calculated as per the following equations (10, 11 & 12):

$$\text{Economic Yield (kg)} = \text{Weight of threshed grains of each microplot ... (10)}$$

$$\text{Biological Yield (kg)} = \text{Weight of harvested crop (shoot \& grains)} + \text{Weight of root... (11)}$$

$$\text{Harvest Index} = \text{Economic Yield} / \text{Biological Yield ... (12)}$$

*Seed germination test*

Seed germination test was also done by following rolled towel method (ISTA rules; Anon, 1985). In this, though the use of counting board, hundred seeds are placed evenly between two pre-soaked (in water for 2 hours) germination papers. These were then rolled carefully, ensuring no excess pressure on seeds, wrapped in a sheet of butter paper and placed (in an upright position) in a germination chamber. Temperature of germination chamber was maintained at 20°C for wheat seeds while at 25°C for paddy seeds and allowed to germinate. Number of germinated seeds, as obtained thorough “First count”, on the fourth day of wheat seed germination and on the fifth day of paddy seed germination experiment were estimated. Number of normally germinated, un-germinated, abnormally germinated and dead seeds was also counted during “Final count” stage i.e. on the eighth day and the fourteenth day of wheat and paddy seed germination experiment, respectively. Ten firm seedlings were carefully removed from the germination paper and their root length & shoot length was taken by scale. After removing the seed from each seedling, these ten seedlings were collectively dried in oven for their dry weight measurement. Finally vigour indices (Abdul-Baki and Anderson, 1973) were estimated as per the following equations (13 & 14):

$$\text{Vigour index - I} = (\text{Dry wt.} \times \% \text{ Germination})... (13)$$

$$\text{Vigour index - II} = \{(\text{Root length} + \text{Shoot length}) \times \% \text{ Germination}\}... (14)$$

It is important to mention that so to break paddy seed dormancy, just after harvesting paddy seeds were kept at 50°C for five days and thereafter subjected to the germination experiment.

#### *Heavy metal analysis*

Both micro-nutrient (viz. Zn, Cu, Fe, Mn) and trace metal (viz. Cd, Cr, Co, Ni, Pb) concentrations were estimated. For this, 0.5 g of ground plant sample was taken in a 150 ml conical flask. To this 15 ml of di-acid mixture (9:4::HNO<sub>3</sub>:HClO<sub>4</sub>) was added and placed on a hot plate maintained at a temperature of 70<sup>0</sup>C. Digested samples were filtered in 100 ml volumetric flask and their volume made up to 100 ml by using deionised water. Thereafter, the concentrations of Fe, Mn and Zn in acid digested samples were determined through an atomic absorption spectrometer (ECIL AAS4141 model) while those of Cu, Ni, Cd, Cr, Co, and Pb were determined by means of a Polarographic TraceLab Analyzer (POL150 model; Wang, 1989).

### **3.4 COMPREHENSIVE RISK ASSESSMENT DUE TO HEAVY / TRACE METALS**

USEPA (1992) has identified following eight pathways for estimating human exposure to pollutants applied to soils through wastewater irrigation.

1. **Wastewater → Soil → Plant → Human**
2. Wastewater → Soil → Human
3. Wastewater → Soil → Plant → Animal → Human
4. Wastewater → Soil → Animal → Human
5. Wastewater → Soil → Airborne particulate → Human
6. Wastewater → Soil → Surface runoff → Surface water → Human
7. Wastewater → Soil → Vadose zone → Groundwater → Human
8. Wastewater → Soil → Atmosphere → Human

In these exposure routes, the first one i.e. food-chain transfer is the primary route of human exposure to environmental pollutants, in which pollutant intake from the consumption of grain, vegetables, root/tuber crops & fruit reaches the human body. The pollutant ingested due to consumption of cereal, vegetable, root/tuber & fruit in turn accounts for 75 % of the total dietary intake of pollutant (Galal-Gorchev, 1991) while the pollutant intakes through drinking water and inhalation are not expected to

exceed 5 -10 % of the average daily intake (ADI, WHO, 1998; USEPA, 1992a & 2000). Thus pollutant intake via food consumption is by far the most significant environmental exposure route for pollutants to reach human body (McKone and Ryan, 1989). Cereals normally accumulate lesser heavy metals than vegetables, but health risk is by far greater due to their higher contribution in diet. When heavy metal enters the body, it first accumulates to the toxic levels in the body tissues & thereafter different organs create many types of problems like cancer.

To quantify these risks and to access “**Food chain transfer**” of heavy metals i.e. the quantity of heavy metal transport/ load/ enrichment from irrigation water to soil, plant tissue, grains & ultimately to human body, a comprehensive risk assessment was done as per the following procedures:

Extent of pollutant transfer from the irrigation water to soil was assessed in terms of the **Pollution Load Index** (PLI, Tomlison et al., 1980) where PLI can be expressed as in the following equation (15):

$$PLI = (CF_1 \times CF_2 \times CF_3 \dots \times CF_n)^{1/n} \dots (15)$$

Where,  $CF_i$  is the **Contamination Factor** (Hakanson et al., 1980) of each pollutant would be obtained as in the following equation (16):

$$CF = C_o^i / C_n^i \dots (16)$$

Where,  $C_o^i$  is mean concentration of a specific pollutant in treated sewage irrigated micro-plots, and  $C_n^i$  is mean concentration of a same pollutant in sewage water irrigated micro-plots. CF value indicates degree of soil contamination, due to each metal, through poor quality water as assessed through the following four categories:

- $CF < 1$             low contamination factor
- $1 \leq CF \leq 3$        moderate contamination factor
- $3 \leq CF < 6$        considerable contamination factor
- $6 \leq CF$             very high contamination factor

While PLI quantifies the overall contamination of soil by the poor quality water by considering all heavy metal effects collectively. Pollution Load Index (PLI) value close to 1 indicates heavy metal loads near the background levels, while values above 1 indicate soil pollution (Cabrera et al., 1999).

Extent of pollutant transfer from soil to plant, in turn, was ascertained through following two indices viz. Bio-concentration factor (BCF) and Enrichment Factor

(EF). Bio-concentration factor (BCF) is an estimate of the plant tissue concentration of each heavy metal and was obtained through the following equation 17, (Barman et al., 2000):

$$\text{BCF} = (\text{Metal concentration in plant tissue} / \text{Metal concentration in rooted soil}) \dots(17)$$

While Enrichment factor (EF) is used to estimate the translocation of each heavy metal in the plant tissue from contaminated soil over sewage water irrigated soil, and was obtained through the following (Buat-Menard & Chesselet, 1979) equation (18):

$$\text{EF} = (\text{BCF of edible part produced from treated sewage microplot}) \div (\text{BCF of edible part produced from sewage water irrigated micro-plot}) \dots (18)$$

According to Sutherland (2000), five contamination categories are generally recognized on the basis of the enrichment factor:

**EF < 2          deficiency to mineral enrichment**

**EF = 2 to 5    moderate enrichment**

**EF = 5 to 20   significant enrichment**

**EF = 20 to 40 very high enrichment**

**EF > 40        extremely high enrichment**

Higher values of enrichment factor (EF) suggest poor retention of metals in soil and/or more translocation in plants. Enrichment factor of heavy metals depends upon bioavailability of metals, which in turn depends upon its concentration in the soil, their chemical forms, difference in uptake capability and growth rate of different plant species (Tinker, 1981).

While pollutant transfer/ translocation between different plant parts was evaluated through the **Translocation Factor (TF)**, (Cui et al., 2004), where TF is the fraction of pollutant concentration in different plant parts w.r.t. its concentration in the roots. High TF value indicates a strong accumulation by crop.

Finally the threat of pollutant contamination, due to untreated and treated waste water irrigations, on the consumer (i.e. human) health was assessed in terms of the **Health Risk Index (HRI)** as per the following equation (19):

$$\text{Health Risk Index (HRI)} = \text{HQ} \times f \dots (19)$$

Where,  $f$  is the consumption probability of contaminated grains and HQ is Hazard Quotient. Pierzynski et al. (2000) evaluated the hazard quotient in the following manner (equation 20):

$$\text{HQ} = (\text{Daily intake of a specific heavy metal}) / (\text{Its Safe limit}) \dots (20)$$

Where, Daily intake of heavy metal (in mg metal/Kg body weight/day) is generally obtained as (Concentration of metal in contaminated food  $\times$  Daily food intake) / (Average body weight). HRI index value  $>1$  is considered unsafe for human health (USEPA, 2002).

The risk by sum of the all heavy metal constituent present in the contaminated grains was analyzed by **Hazard Index** (Huang et al. 2007; eq. 21), which is the measure of the potential risk of adverse health effects from a mixture of heavy metal constituents in the contaminated grains. When the hazard index exceeds 1.0 there is concern for potential health effects.

$$\text{HI} = \sum_{i=1}^n \text{HRI} \dots (21)$$

Daily food intake pattern of the exposed individual is represented by the global diet (Galal-Gorchev, 1991) which considers daily intake of grains/cereals of about 0.405 kg (on fresh weight basis where the fresh weight to dry weight conversion factor is 0.90 for grains/cereals). The dietary intake of pollutants normally does not exceed 50 % of the Provisional Tolerable Weekly Intake (PTWI), except for individuals who are exposed through occupational activities or are resident near a pollution point source (WHO, 1998; USEPA, 1992a & 2000). Hence for this analysis, the consumption probability of contaminated grains was considered as 25%. Further, the average body weight of an adult was considered as 70kg while the oral reference dose or safe daily intake quantity of different heavy metals, as per USEPA (1997 & 2002), was considered to be Cu (0.04mg/kg/day), Zn (0.3 mg/kg/day), Cd (0.001 mg/kg/day), Pb (0.004 mg/kg/day), Ni (0.02 mg/kg/day), Cr (1.5 mg/kg/day), Fe (0.25 mg/kg/day) and Mn (0.14 mg/kg/day), Co (1.6 mg/kg/day; FSA, 2003).

## RESEARCH PAPER-I

### To assess impact of IARI wetland treated sewage water applications on the soil nutrient and heavy metal load at the experimental sewage plot site

---

#### Abstract

Eighteen micro-plots (2.7 m<sup>2</sup> area) at the sewage plot site of IARI (New Delhi), with Rice-Wheat cropping system, were irrigated with subsurface flow constructed wetland treated sewage waters for two years. Continuous application of treated sewage waters, for 2-years, in place of untreated sewage water applications (*business-as-usual*) at the project site resulted in significant reduction in soil potassium concentration from an initial (beyond permissible) levels of (219.19 ± 12.66 mg/kg) to (125.27 ± 10.57 mg/kg). Soil nitrate and phosphate concentrations also decreased by about 88% and 38%, respectively thereby showing a significant impact of water treatment on the soil nutrient load. Soil total and bio-available nickel (Ni), lead (Pb), and iron (Fe) concentrations also decreased significantly. Soil bio-available chromium decreased from an initial level of (5.71 ± 0.88 mg/kg) to (1.57 ± 0.07 mg/kg) after two years. However, there was no change in the soil total chromium (Cr) level, due to its more than (1.7 times) permissible concentrations in the historically sewage water irrigated soils of the experimental area. Soil bio-available zinc concentrations changed insignificantly from (6.38 ± 0.55 mg/kg) to (5.81 ± 0.51 mg/kg) and cadmium and copper concentrations were found to be below detection limits throughout the research period. Thus, continuous irrigations with treated sewage waters could lead to significant soil pollutant load reductions and no depletion in soil micro-nutrient concentrations as well as any adverse effects due to soil electrical conductivity and exchangeable sodium percentage, which remained within safe limits.

#### 4.1 Introduction

Water scarcity is one of the global problems. Increasing population, rapid urbanization and industrialization along with climate change are causing significant impact on water demand, quality and supply. Several adaptation and mitigation strategies have been developed and evaluated to cope up with these problems. Amongst these, wastewater reuse in agriculture seems to be the most promising strategy. Globally, in at least 44 countries, about 5, 25,000 ha land area is subjected to reclaimed water irrigation (FAO, 2010) at a discharge rate of over 15 Mm<sup>3</sup>/d (Jiménez

and Asano, 2008). In developing countries due to the lack of awareness and low cost/low energy demanding treatment facilities, unplanned raw sewage application is increasing. For instance, in terms of area, India (with over 73,000 ha area) occupies third position in the use of untreated wastewater for (Scott et al. 2010) for growing different crops and aquaculture (Strauss and Blumenthal, 1990). Most of these wastewater irrigated areas are situated along rivers, flowing through rapidly growing cities such as Delhi, Kolkata, Coimbatore, Hyderabad, Indore, Kanpur, Patna, Vadodara, Varanasi, Dharward, etc.

Wastewater re-use fulfils the demand of water in scarce areas and its high nutrient content supplements the fertilizer dose. As a resulting several rainfed farmers are rapidly converting to wastewater irrigators. It has been estimated that wastewater use at the rate of 3000 m<sup>3</sup> per hectare can supplement N (48-186 kg/ha), P (12-72 kg/ha), K (6-607 kg/ha), Ca (54-624 kg/ha), Mg (27-330 kg/ha) and Na (81-546 kg/ha; Lzarova and Bahri, 2005). However, unplanned and untreated or improperly treated wastewater application increases problems of salinity, sodicity and heavy metal accumulation in soil and groundwater while excess nutrient with agricultural drainage degrades surface water quality and aquatic life.

Nitrogen is present in wastewater as nitrate, ammonia, organic nitrogen and nitrite. Most plants absorb nitrates (National Research Council, 1996). Only 50% of ammonia and 30% of organic nitrogen are assimilated by plants, as rest is lost during transformation through several mechanisms such as volatilization (Girovich, 1996). Nitrates are very soluble in water and so washed out very easily depending on irrigation rate, soil characteristics and nitrogen content of wastewater. Phosphorus, though bio-available, is often scarce in soil. Wastewater normally contains low amount of phosphorus and thus can be applied over long periods of time (Degens et al., 2000) without impacting the environment negatively (Girovich, 1996). Potassium on the other hand, though present in high concentrations in soil, is generally not bio-available. As wastewater contains low potassium concentrations therefore even this does not normally cause any negative environmental impacts (Mikkelsen & Camberato, 1995).

Salinity in the form of sodium can directly affect soil properties through the phenomena of swelling and dispersion (Halliwell et al., 2001). These effects occur as sodium, which is a positively charged cation, interacts with the negatively charged layers (known as platelets) of clay particles. As sodium concentrations increase, the

electrophoretic mobility of the clay platelets increases resulting in swelling/dispersion of the clay particles and thus impacting soil permeability (Halliwell et al., 2001). Along with sodium, presence of suspended solids (Magesan et al., 2000) and other nutrients which cause excessive growth of microorganisms in the soil (Magesan et al., 1999) or interaction of dissolved organic matter with the soil profile (Tarchitzky et al., 1999) causes reduction of hydraulic conductivity of soil. This can impact on the ability of water to infiltrate into the soil profile and thus, reduces the water availability to irrigated crops. Concentration of carbonates and bicarbonates above 500 mg/l can also cause soil deflocculation.

Among numerous hazardous materials, heavy metals are extremely persistent in the environment. Further they are non-biodegradable and non-thermo-degradable (Cao and Hu, 2000). Long-term use of wastewaters on agricultural lands often results in their build-up to the elevated levels in the arable layer of soils (Rattan et al., 2002). Extent of build-up of metals in waste water-irrigated soils depends on the period of its application (Bansal et al., 1992; Palaniswami and Sree Ramulu, 1994). Contamination of soils and crops due to wastewater irrigation are widely reported from different parts of the world. For example it has been estimated that about 45% of wastewater irrigated areas in China are contaminated with very alarming levels of heavy metals. Not only in China, this problem is emergent in several other countries like Germany, France and India as well (Ingwersen and Streck, 2006; Dere et al., 2006; Singh and Kumar, 2006). In several studies, it is established that solubility of metals in soils mainly depends on soil pH and organic carbon (Jopony and Young, 1994; Hough et al., 2003; Tye et al., 2003). When the capacity of the soil to retain heavy metals is reduced due to repeated use of domestic wastewater, soil can release heavy metals into ground water or soil solution available for plant uptake (Sharma et al., 2007). Generally a metal will not be absorbed by the plants unless it first reaches a threshold concentration in the soil. Metals are bound to soil with pH above 6.5 and high organic matter content. At lower than 6.5 pH, organic matter is consumed and metals become mobile and can thus be readily absorbed by the crops and contaminate water bodies (WHO, 2006). Cadmium, copper, molybdenum, nickel and zinc are frequently present in wastewaters and can be easily mobilized and absorbed by the plants. In fact long term high metal concentrations are known to pose serious threats to the stabilization and health of soil ecosystems.

Wastewater use in agriculture also adds soil organic matter (SOC) which enriches the humic content that increases soil moisture, retains metals (through cationic exchange and the formation of organometallic compounds) and enhances microbial activity. Some estimates show that such an increase in the SOC by 0.01% can lead to carbon sequestration equivalent to the annual increase in the atmospheric CO<sub>2</sub>-C (Lal et al., 1998). Thus long-term application of sewage effluents is a carbon-building/sequestering and soil quality sustaining practice and thus plays a major role in nutrient cycling. However, there are many studies that also illustrate their negative impacts on the soil microbial biomass, microbial structure, microbial diversity and thus enzyme activities and C/ N mineralization.

Among different wastewater treatment technologies constructed wetlands are now a day becoming one of the more focussed research areas in the world. Due to less demand for energy, skilled persons and optimum treatment level, its scope for safe agricultural wastewater disposal is more in the developing countries. However, its evaluation in the actual field conditions is necessary before its large scale application/ adoption. One such wetland technology based pilot sewage water treatment plant (of 1500 LPD capacity) is in operation since November 2009, at the sewage plot site of Indian Agricultural Research Institute (IARI) farmlands. Thus, the main objective of the present study was to assess impact of the aforementioned wetland - treated sewage water applications on the nutrient and the heavy metal load of the experimental sewage plot site.

#### **4.2 Material and methods**

A pilot scale vertical sub-surface flow (VSSF) constructed wetland system was developed and sited near a domestic sewage water drain on a 350 m<sup>2</sup> land area (28°38'21.3" N and 77°08'56.5" E) within the Seed Production Unit (SPU) farm of the Indian Agricultural Research Institute (IARI). Sewage water generated from the IARI micro watershed containing moderate organic load, lab chemical compounds and heavy metals was treated and applied in eighteen Paddy-Wheat cultivated micro-plots, each having size 1.8m × 1.5m of area (2.7 m<sup>2</sup>), adjacent to the treatment plant (mesocosm) for two years (including my one year experiment). The experimental field has sandy loam type soil and a pre-contaminated history with sewage. The six treatments viz. *Typha latifolia* (Cattail) treated water (TW), *Phragmites karka* (Reed) treated water (PW), *Acorus calamus* (Vacha) treated water (VW), Control (No

vegetation) treated water (CW), raw Sewage water (SW) and Ground water (GW) irrigation micro-plots were arranged in randomized block design (RBD) in three replicates.

Wheat (Variety HD-2894) was grown with three irrigations (each of 2 cm or 54 litres) in a month and Paddy (Pusa Basmati-1121) with irrigation (each of 2 cm or 54 litres) at each alternate day. Initial basal fertilizer dose was also applied in each micro-plot @ 35g Urea/plot, 17.5g DAP/plot & 17.5g MOP/plot in both Wheat and Paddy crop. The water sampling dates were coincided with the field-irrigation dates. The effluent water samples from each mesocosm and the sewage and groundwater samples were collected thrice every month, at approximately 20 days interval, during Rabi (Nov. – March) season and on alternate days during Kharif (June – Oct.) season. Monthly suitable composite samples were prepared in 500 ml capacity plastic bottles. The pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), oxidation-reduction potential (ORP), salinity and temperature of the water samples were determined by HORIBA U-51 probe while turbidity by turbidimeter (Hach-2100P-Turbidimeter) and then water samples were immediately transferred to the laboratory and stored in a refrigerator at 4°C until further analysis of the remaining physico-chemical parameters. The same water samples were also collected in 100 ml capacity plastic bottles. These were immediately acidified with 1 N HNO<sub>3</sub> for heavy metal analysis.

In case of wheat, the soil sampling was done one before sowing (Nov. 2010), one at mid-crop season (Feb. 2011) and one at the end of the wheat crop season (April 2011). While in case of paddy, the last soil sampling of wheat season was assumed as the beginning sample for paddy season and final soil sampling was done at the end of the paddy crop season (Nov. 2011). The top (0-30 cm) soil layer samples from each micro-plot were collected randomly from five spots by a core sampler to form a bulk (500 g) soil sample. The soil samples were packed in pre-marked polyethylene bags and immediately transferred to the laboratory, where these were processed i.e. air dried, crushed, ground and sieved through a 2-mm sieve.

pH and Electrical conductivities (EC) of 1:5 (Soil: Distilled water) extracts were determined by means of a pre-calibrated pH and conductivity meters (Jackson, 1973). Carbonate (CO<sub>3</sub><sup>2-</sup>) bicarbonate (HCO<sub>3</sub><sup>-</sup>) and chloride (Cl<sup>-</sup>) concentrations (meq/l) of influent/ effluent water samples were determined titrimetrically (Richards, 1954). Calcium (Ca) and Magnesium (Mg) concentrations (meq/L) by Versenate

(Ethylene-Di-Amine-Tetra-Acetic Acid, EDTA) titration method (Jackson, 1973) and fluoride concentrations (F, ppm) by SPADNS colorimetric method (Bellack & Schouboe, 1968). Nitrate concentrations (NO<sub>3</sub>, ppm) were determined by ultraviolet spectroscopic screening method; sulphate concentrations (SO<sub>4</sub>, ppm) by turbidimetric method (Clesceri *et al.*, 1998); phosphate concentrations (PO<sub>4</sub>, ppm) in water (through amino acid method) and soil (through Olsen's method; Olsen *et al.*, 1954), by means of a UV-VIS spectrophotometer (Hach DR-5000). Sodium (Na) and potassium (K) concentrations were determined by flame photometer (Elico CL-361, Jackson, 1973) and Oxidisable Organic Carbon (OOC) through chromic acid oxidation method (Walkley and Black, 1934). Soil bio-available (DTPA extractable) and total heavy metal (digested using di-acid mixture i.e. HNO<sub>3</sub>:HClO<sub>4</sub>:: 9:4) concentrations viz. Fe, Mn, Zn were determined through an atomic absorption spectrometer (ECIL AAS4141 model) while Ni, Cd, Cr, Co, Cu and Pb by means of a Polarographic Trace Lab Analyzer (POL150 model; Wang, 1989).

Extent of pollutant transfer from irrigation water to soil was assessed in terms of the Pollution Load Index (PLI, Tomlison *et al.*, 1980). PLI quantifies the overall contamination of soil, due to poor quality water applications, in terms of say all heavy metals. Pollution Load Index (PLI) value close to 1 indicates pollution (or heavy metal) load close to background level, while PLI above 1 indicates soil pollution more than background levels (Cabrera *et al.*, 1999). As the soil of the experimental site was earlier contaminated due to raw sewage application therefore, in this study the CF and PLI were calculated in the following manner:

$$PLI = (CF_1 \times CF_2 \times CF_3 \dots \times CF_n)^{1/n}$$

where, CF<sub>i</sub> is the Contamination Factor (Hakanson, 1980) of each pollutant and was obtained as:

$$CF = C_o^i / C_n^i$$

where, C<sub>o</sub><sup>i</sup> is mean concentration of a specific pollutant in treated sewage irrigated micro-plot, and C<sub>n</sub><sup>i</sup> is mean concentration of the same pollutant in the sewage water irrigated micro-plot.

### 4.3 Results and Discussions

During the study period, temperature, pH and dissolved oxygen levels of the untreated sewage waters were observed to be within the permissible limits (i.e. 30°C, 8.0 and 5mg/l) while turbidity ( $67.01 \pm 42.57$  NTU) was found to be mostly beyond permissible level (50 NTU) (Figure 4.1c). In comparison to these, the wetland treated sewage waters were associated with significantly (40 to 74%) lower turbidity (17.70 NTU), nitrate (2.78 ppm, Figure 4.4a), phosphate (11.75 ppm, Figure 4.4b) and potassium (9.39 ppm, Figure 4.6b) levels. Sulphate, fluoride, EC and TDS levels (Figure 4.4c, 4.2a, 4.3b and 4.3a) of the irrigation waters were also in general found to be within the permissible limits. Amongst heavy metals, chromium, manganese and iron were the key contaminants (Figure 4.10, 4.8a and 4.8c) that were present in their more than permissible levels. Rest metals viz. nickel, lead, zinc (Figure 4.9a, 4.9b and 4.8b), though present in appreciable amounts, were within their safe limits.

Continuous application of treated sewage waters, on the historically sewage water irrigated site, revealed that electrical conductivity of soil (EC<sub>w</sub>, Figure 4.11a) after a slight increase from an initial level  $0.32 \pm 0.02$  dS/m in Nov. 2009 to  $0.73 \pm 0.16$  dS/m in Nov. 2010 finally reverted back to its initial level of around  $0.29 \pm 0.08$  dS/m in Nov. 2011. Exchangeable Sodium Percentage (ESP, Figure 4.11b) value also increased from an initial level of  $1.72 \pm 0.23\%$  to  $5.44 \pm 2.39\%$  during first year and finally reverted back to  $1.66 \pm 0.52$  in the second year. However, treated sewage water applications could significantly reduce excessive soil potassium concentrations (Figure 4.11c) from an initial (beyond permissible) level of  $219.19 \pm 12.66$  mg/kg to  $125.27 \pm 10.57$  mg/kg. Similarly soil nitrate (Figure 4.11d) and phosphate concentrations (Figure 4.11e) also seem to be decreasing from initial levels of  $22.44 \pm 1.95$  mg/kg and  $28.74 \pm 1.36$  mg/kg respectively to  $2.60 \pm 1.61$  mg/kg and  $17.97 \pm 1.73$  mg/kg, respectively thereby showing a significant impact of water treatment on the soil nutrient load.

Within 2-years of treated sewage water applications, soil bio-available pool of nickel (Figure 4.13a) decreased from an initial unsafe level of  $1.86 \pm 0.48$  mg/kg to a safe level of  $0.30 \pm 0.11$  mg/kg. Soil bio-available chromium concentrations (Figure 4.13b) also decreased from an initial level of  $5.71 \pm 0.88$  mg/kg to  $1.57 \pm 0.07$  mg/kg after two years. Though it was still above its permissible level yet it was mostly in Cr<sup>3+</sup> form and very less in toxic, Cr<sup>6+</sup> form thereby illustrating a positive impact of treated wastewater applications. Lead bio-available concentrations (Figure 4.13c) were also initially high ( $7.03 \pm 1.32$  mg/kg) and beyond safe limit (5 mg/kg) and

decreased to  $(3.33 \pm 0.79 \text{ mg/kg})$ , in 2 years of the experimental period. Similarly manganese and iron concentrations (Figure 4.12a and 4.12c), though still far beyond their permissible levels (0.2 ppm and 5 ppm, respectively), could be significantly reduced from  $(27 \pm 1.66 \text{ mg/kg})$  and  $(68.77 \pm 1.05 \text{ mg/kg})$  levels, respectively to  $(4.69 \pm 0.60 \text{ mg/kg})$  and  $(31.45 \pm 3.74 \text{ mg/kg})$ , respectively during 2-years. Soil bio-available zinc concentration (Figure 4.12b) changed insignificantly from  $(6.38 \pm 0.55 \text{ mg/kg})$  to  $(5.81 \pm 0.51 \text{ mg/kg})$  and cadmium and copper concentration were found to be below detection limits throughout the research period.

Though total initial soil heavy metal load w.r.t. nickel was observed to be within permissible limit (75 mg/kg) yet, due to short term irrigations with treated wastewaters on the historically contaminated site, its level decreased significantly from  $(42.79 \pm 15.58 \text{ mg/kg})$  to  $(7.93 \pm 1.12 \text{ mg/kg})$ . This was also observed to be the case with lead contamination, which was initially higher than the safe limit (84 mg/kg) and eventually decreased from an initial level of  $104.51 \pm 21.17 \text{ mg/kg}$  to  $17.32 \pm 3.44 \text{ mg/kg}$  during the experimental period. During *kharif* season, (total) soil nickel, lead and cobalt contamination (Table 4.2, Figure 4.16a and 4.16b) of the experimental micro-plots irrigated with treated wastewaters discharged from *Typha latifolia* based wetland systems was about 17, 30 and 61% lower than those for the sewage water irrigated micro-plots. However, these seem to be reverting back to their (latest within permissible) background levels for the sewage water irrigated micro-plots during *rabi* season (Table 4.4, Figure 4.16c and 4.16d). During *rabi* season, due to differential pollutant removal efficiency of the varied (so far not harvested) wetland systems, micro-plots receiving treated wastewaters from unplanted mesocosms and *Typha* sp. / *Acorus* sp. based wetland systems were found to be associated with significantly lower soil Pb and Ni-contaminations, respectively than those receiving treated wastewaters from other wetland systems. This could also be illustrated by the metal pollution load indices (PLIs) for the *kharif* (Table 4.5 and Figure 4.17c) and *rabi* seasons (Table 4.5 and Figure 4.17d). Thus continuous application of treated wastewaters, with significantly lower nutrient and metal concentrations, could considerably reduce pollution load in the historically sewage water irrigated soils.

However, concentration of total soil chromium (Figure 4.15b), that was about 1.7 times more than its permissible limit of (150 mg/kg) in the historically sewage water irrigated soils, remained unchanged throughout the experimental period thereb

Table 4.1: Contamination factor of different metals with respect to ground water ( $CF_{GW}$ ) for paddy

<b>SAMPLE</b>	<b>Ni</b>	<b>Co</b>	<b>Pb</b>	<b>Cr</b>	<b>Mn</b>	<b>Zn</b>	<b>Fe</b>
<b>TW</b>	$1.52 \pm 0.15$	$1.67 \pm 1.70$	$0.96 \pm 0.10$	$1.19 \pm 0.04$	$1.00 \pm 0.06$	$1.57 \pm 0.43$	$1.00 \pm 0.01$
<b>PW</b>	$1.48 \pm 0.79$	$4.21 \pm 4.33$	$0.96 \pm 0.18$	$1.05 \pm 0.08$	$1.03 \pm 0.17$	$0.99 \pm 0.10$	$1.00 \pm 0.03$
<b>VW</b>	$2.01 \pm 0.53$	$2.94 \pm 3.02$	$0.41 \pm 0.41$	$1.00 \pm 0.01$	$1.04 \pm 0.04$	$1.10 \pm 0.19$	$0.99 \pm 0.02$
<b>CW</b>	$2.16 \pm 0.87$	$4.36 \pm 6.04$	$1.00 \pm 0.51$	$1.05 \pm 0.12$	$0.98 \pm 0.11$	$1.20 \pm 0.32$	$0.99 \pm 0.02$
<b>SW</b>	$1.87 \pm 0.41$	$3.73 \pm 3.07$	$1.44 \pm 0.29$	$1.00 \pm 0.01$	$0.98 \pm 0.14$	$1.07 \pm 0.26$	$0.99 \pm 0.01$

Table 4.2: Contamination factor of different metals with respect to sewage water ( $CF_{SW}$ ) for paddy

<b>SAMPLE</b>	<b>Ni</b>	<b>Co</b>	<b>Pb</b>	<b>Cr</b>	<b>Mn</b>	<b>Zn</b>	<b>Fe</b>
<b>TW</b>	$0.83 \pm 0.18$	$0.39 \pm 0.10$	$0.70 \pm 0.22$	$1.19 \pm 0.04$	$1.03 \pm 0.12$	$1.47 \pm 0.20$	$1.01 \pm 0.02$
<b>PW</b>	$0.76 \pm 0.33$	$1.06 \pm 0.28$	$0.70 \pm 0.26$	$1.04 \pm 0.09$	$1.05 \pm 0.03$	$0.97 \pm 0.26$	$1.00 \pm 0.02$
<b>VW</b>	$1.09 \pm 0.29$	$0.80 \pm 0.31$	$0.89 \pm 0.14$	$0.99 \pm 0.01$	$1.06 \pm 0.13$	$1.04 \pm 0.10$	$1.00 \pm 0.01$
<b>CW</b>	$1.17 \pm 0.47$	$0.96 \pm 0.69$	$0.67 \pm 0.22$	$1.05 \pm 0.11$	$1.00 \pm 0.07$	$1.15 \pm 0.32$	$1.00 \pm 0.01$
<b>GW</b>	$0.55 \pm 0.11$	$0.50 \pm 0.48$	$0.72 \pm 0.15$	$1.00 \pm 0.01$	$1.03 \pm 0.15$	$0.98 \pm 0.27$	$1.01 \pm 0.01$

Table 4.3: Contamination factor of different metals with respect to ground water ( $CF_{GW}$ ) for wheat

<b>SAMPLE</b>	<b>Ni</b>	<b>Pb</b>	<b>Cr</b>	<b>Mn</b>	<b>Zn</b>	<b>Fe</b>
<b>TW</b>	$0.66 \pm 0.05$	$2.10 \pm 1.05$	1.00	$1.01 \pm 0.04$	$1.08 \pm 0.08$	$0.99 \pm 0.04$
<b>PW</b>	$1.12 \pm 0.62$	$3.03 \pm 0.83$	1.00	$1.12 \pm 0.10$	$1.01 \pm 0.06$	$0.99 \pm 0.02$
<b>VW</b>	$0.64 \pm 0.11$	$1.88 \pm 0.45$	1.00	$0.98 \pm 0.05$	$1.00 \pm 0.05$	$0.98 \pm 0.02$
<b>CW</b>	$0.98 \pm 0.29$	$1.58 \pm 0.44$	1.00	$1.08 \pm 0.06$	$1.07 \pm 0.08$	$1.01 \pm 0.03$
<b>SW</b>	$0.66 \pm 0.17$	$1.48 \pm 0.33$	1.00	$1.08 \pm 0.08$	$0.99 \pm 0.12$	$1.00 \pm 0.01$

Table 4.4: Contamination factor of different metals with respect to sewage water ( $CF_{SW}$ ) for wheat

<b>SAMPLE</b>	<b>Ni</b>	<b>Pb</b>	<b>Cr</b>	<b>Mn</b>	<b>Zn</b>	<b>Fe</b>
<b>TW</b>	$1.04 \pm 0.17$	$1.52 \pm 1.02$	1.00	$0.94 \pm 0.04$	$1.11 \pm 0.21$	$0.99 \pm 0.03$
<b>PW</b>	$1.63 \pm 0.53$	$2.04 \pm 0.11$	1.00	$1.05 \pm 0.17$	$1.04 \pm 0.17$	$0.99 \pm 0.01$
<b>VW</b>	$0.99 \pm 0.08$	$1.27 \pm 0.05$	1.00	$0.91 \pm 0.06$	$1.02 \pm 0.16$	$0.98 \pm 0.01$
<b>CW</b>	$1.59 \pm 0.73$	$1.06 \pm 0.06$	1.00	$1.00 \pm 0.03$	$1.10 \pm 0.19$	$1.01 \pm 0.02$
<b>GW</b>	$1.59 \pm 0.35$	$0.70 \pm 0.16$	1.00	$0.93 \pm 0.07$	$1.02 \pm 0.12$	$1.00 \pm 0.01$

Table 4.5: Pollution Load Index with respect to sewage water (PLI<sub>SW</sub>) of wheat and paddy

	Wheat		Paddy	
	Season-I	Season-II	Season-I	Season-II
<b>TW</b>	1.17 ± 0.11	1.06 ± 0.14	1.05 ± 0.10	0.87 ± 0.02
<b>PW</b>	1.23 ± 0.08	1.23 ± 0.08	0.99 ± 0.11	0.91 ± 0.08
<b>VW</b>	1.15 ± 0.10	1.02 ± 0.02	0.98 ± 0.08	0.97 ± 0.11
<b>CW</b>	1.04 ± 0.04	1.10 ± 0.08	1.04 ± 0.10	0.95 ± 0.23
<b>GW</b>		1.00 ± 0.08	1.02 ± 0.12	0.76 ± 0.11

Table 4.6: Pollution Load Index with respect to ground water (PLI<sub>GW</sub>) of wheat and paddy

SAMPLE	PLI <sub>GW</sub>	
	Paddy	Wheat
<b>TW</b>	1.16 ± 0.15	1.05 ± 0.09
<b>PW</b>	1.23 ± 0.29	1.23 ± 0.18
<b>VW</b>	1.29 ± 0.23	1.02 ± 0.08
<b>CW</b>	1.29 ± 0.44	1.09 ± 0.01
<b>SW</b>	1.34 ± 0.20	1.00 ± 0.09

indicating no (short term) chromium de-contamination of the treated wastewater irrigated soils. Cadmium and copper were undetected and thus well within their safe limits throughout the research period.

Total iron concentration (Figure 4.14c) was also initially higher than its permissible limit (5000 mg/kg) and dropped to  $(4539.07 \pm 11.82 \text{ mg/kg})$  from an initial level of  $(6931.33 \pm 144.51 \text{ mg/kg})$  due to continuous application of treated wastewaters. Soil manganese concentration (Figure 4.14a), on the other hand was within safe limit (500 mg/kg) throughout the research period and changed insignificantly between  $(238.63 \pm 66.08 \text{ mg/kg})$  to  $(301.47 \pm 7.84 \text{ mg/kg})$ . Similarly zinc level (Figure 4.14b) was also within its safe limit (300 mg/kg) and fluctuated mostly between  $(158.11 \pm 60.89 \text{ mg/kg})$  during the experimental period. This could also be re-confirmed by the soil Fe, Mn and Zn concentration factors (Figure 4.16a, 4.16b, 4.16c and 4.16d) of the micro-plots receiving treated wastewaters, during both *kharif* and *rabi* seasons. The investigation thus indicated no depletion of soil micro-nutrients in non-wastewater irrigated soils.

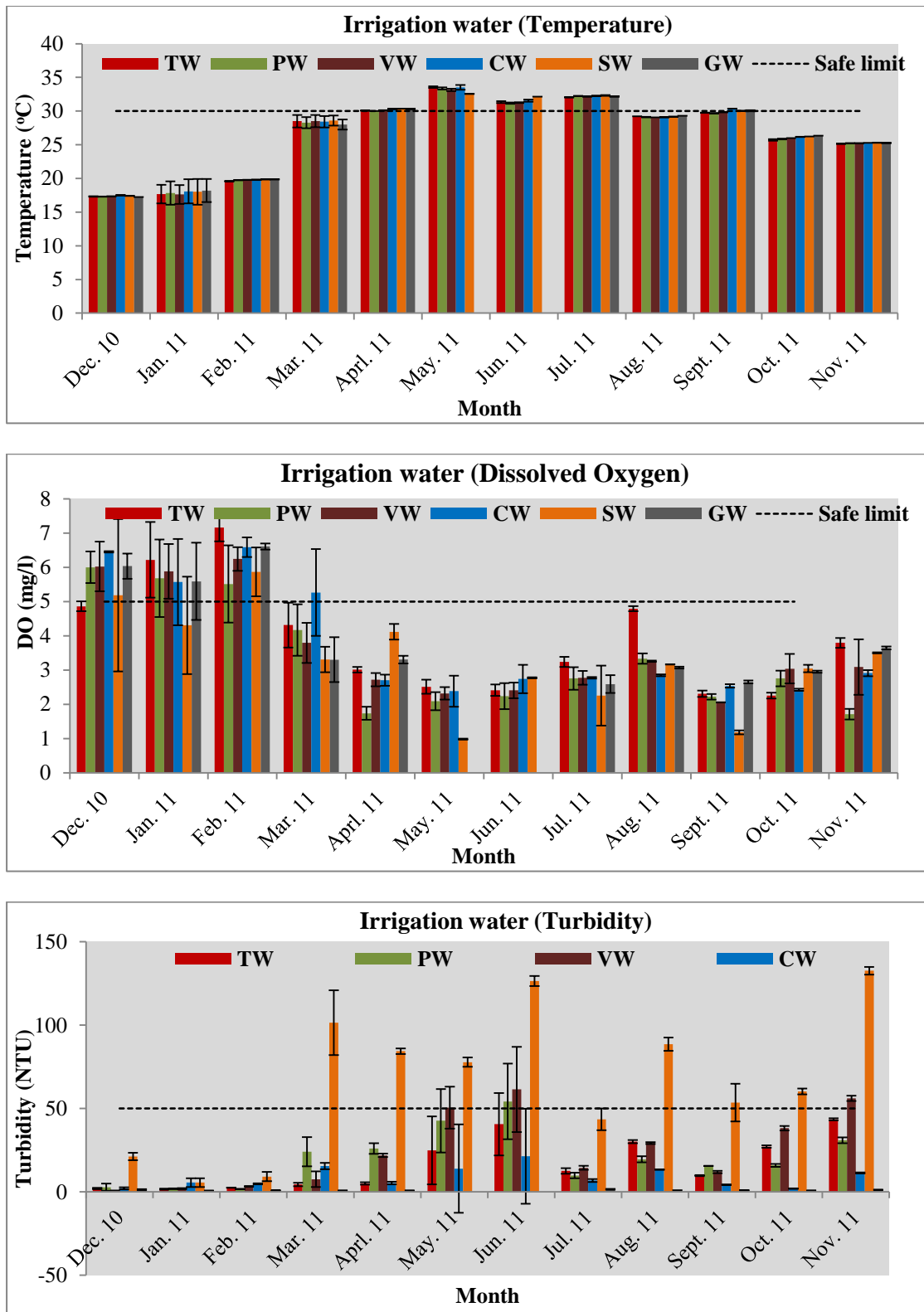


Figure 4.1: Monthly variation in (a) Temperature, (b) Dissolved Oxygen and (c) Turbidity in different irrigation water

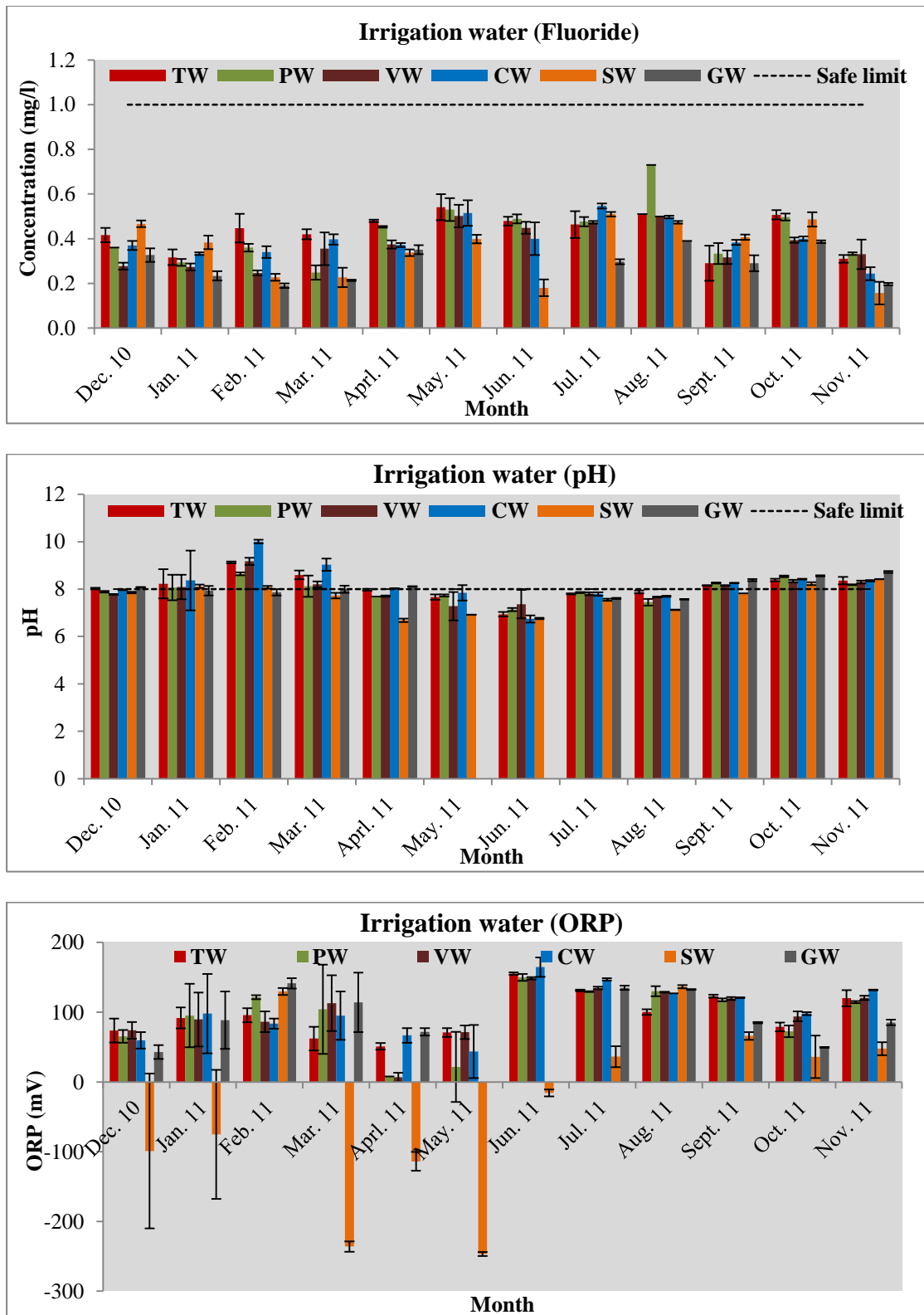


Figure 4.2: Monthly variation in (a) Fluoride (b) pH and (c) Oxidation Reduction Potential value in different irrigation water

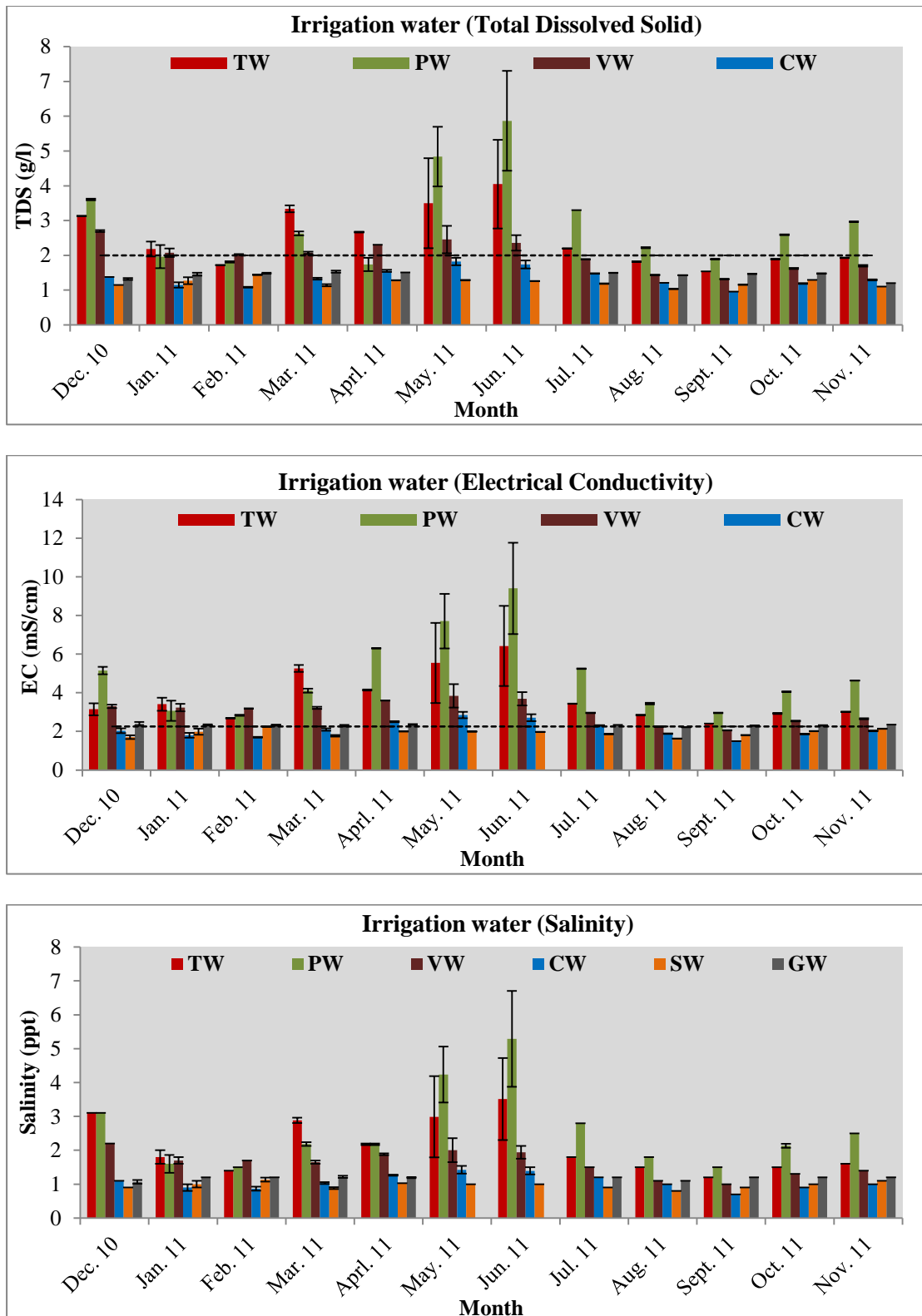


Figure 4.3: Monthly variation in (a) Total Dissolved Solid, (b) Electrical Conductivity and (c) Salinity in different irrigation water

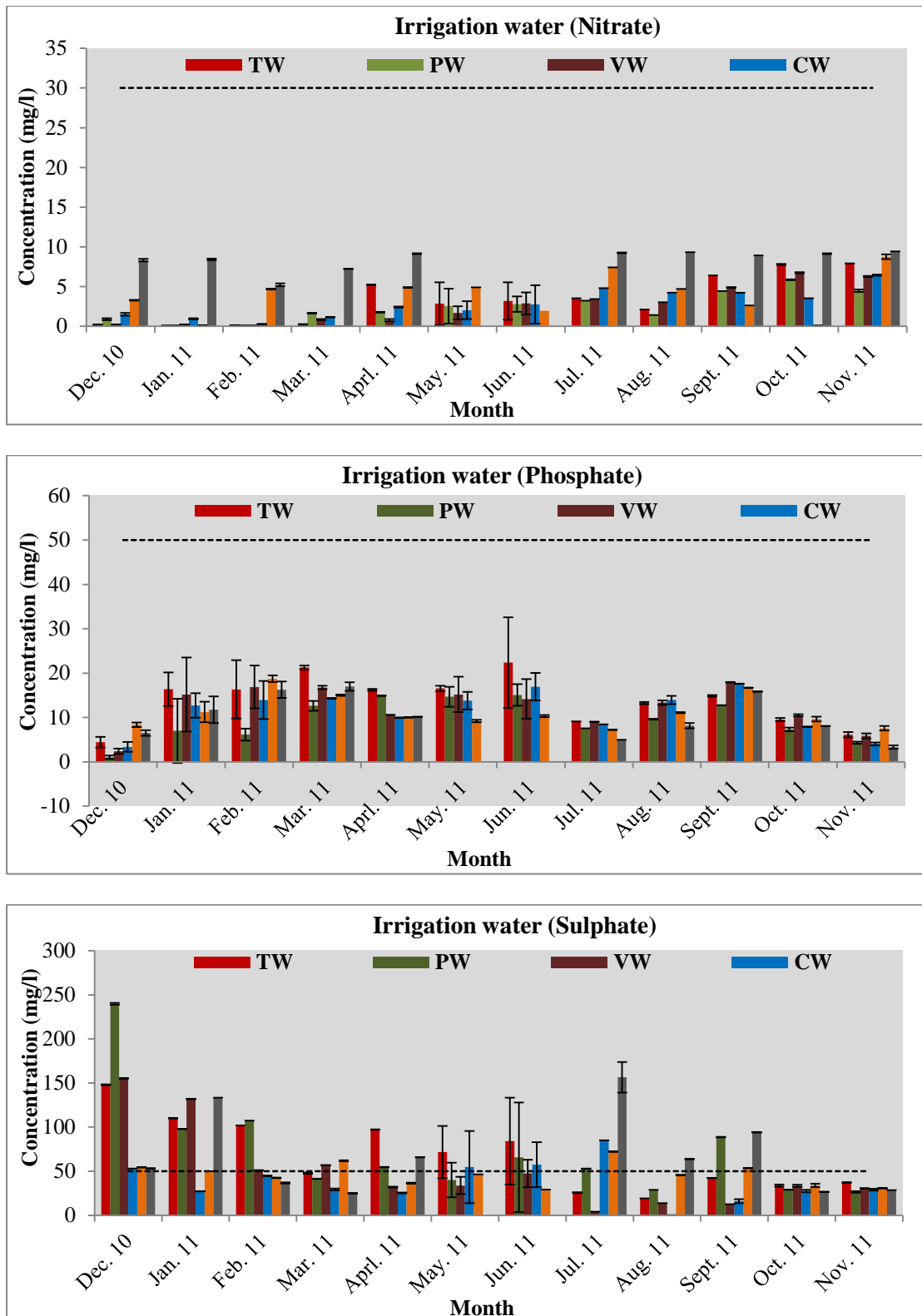


Figure 4.4: Monthly variation in (a) Nitrate, (b) Phosphate and (c) Sulphate concentration in different irrigation water

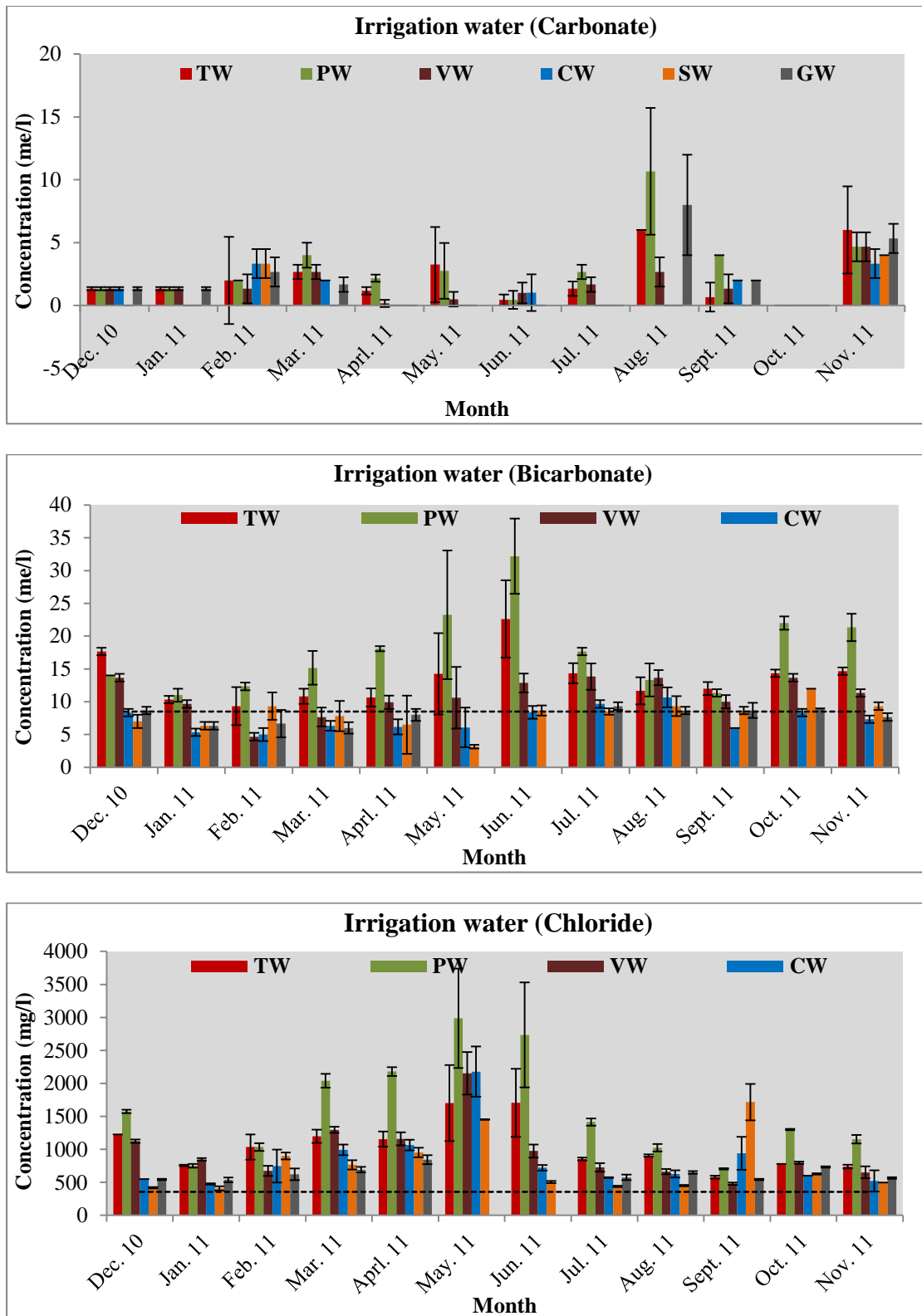


Figure 4.5: Monthly variation in (a) Carbonate, (b) Bi-carbonate and (c) Chloride concentration in different irrigation water

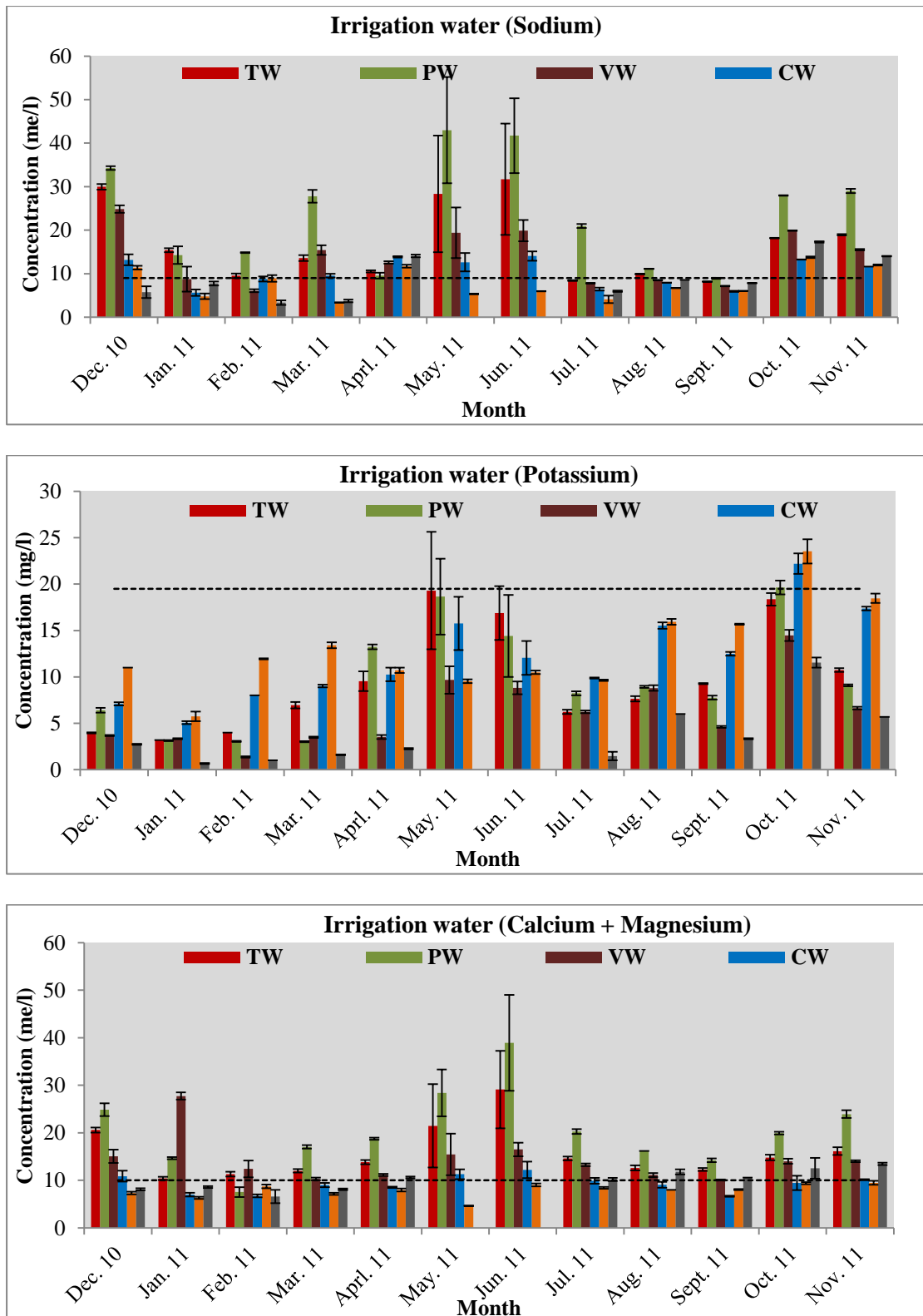


Figure 4.6 Monthly variation in (a) Sodium, (b) Potassium and (c) Calcium and Magnesium concentration in different irrigation water

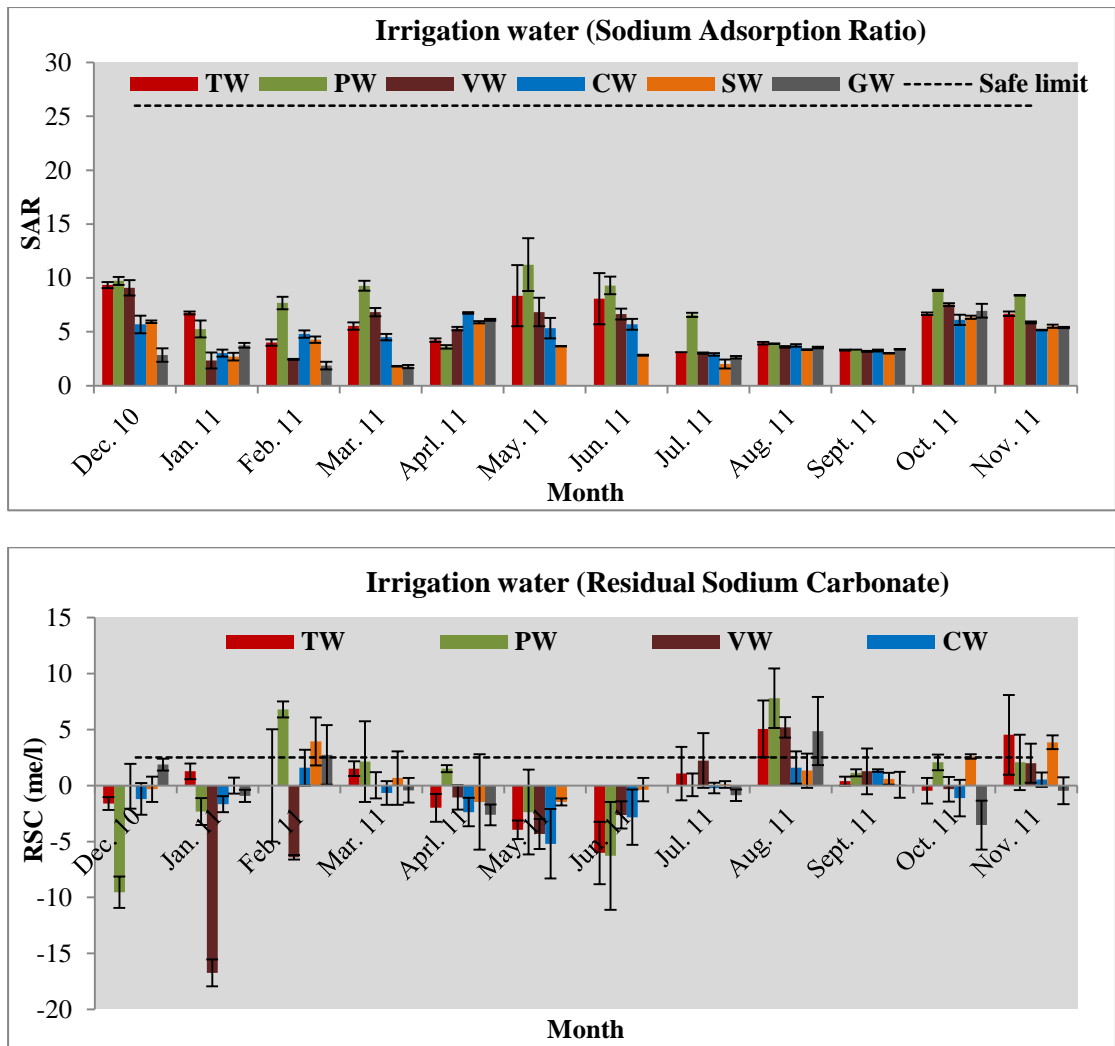


Figure 4.7: Monthly variation in (a) Sodium Adsorption Ratio and (b) Residual Sodium Carbonate value in different irrigation water

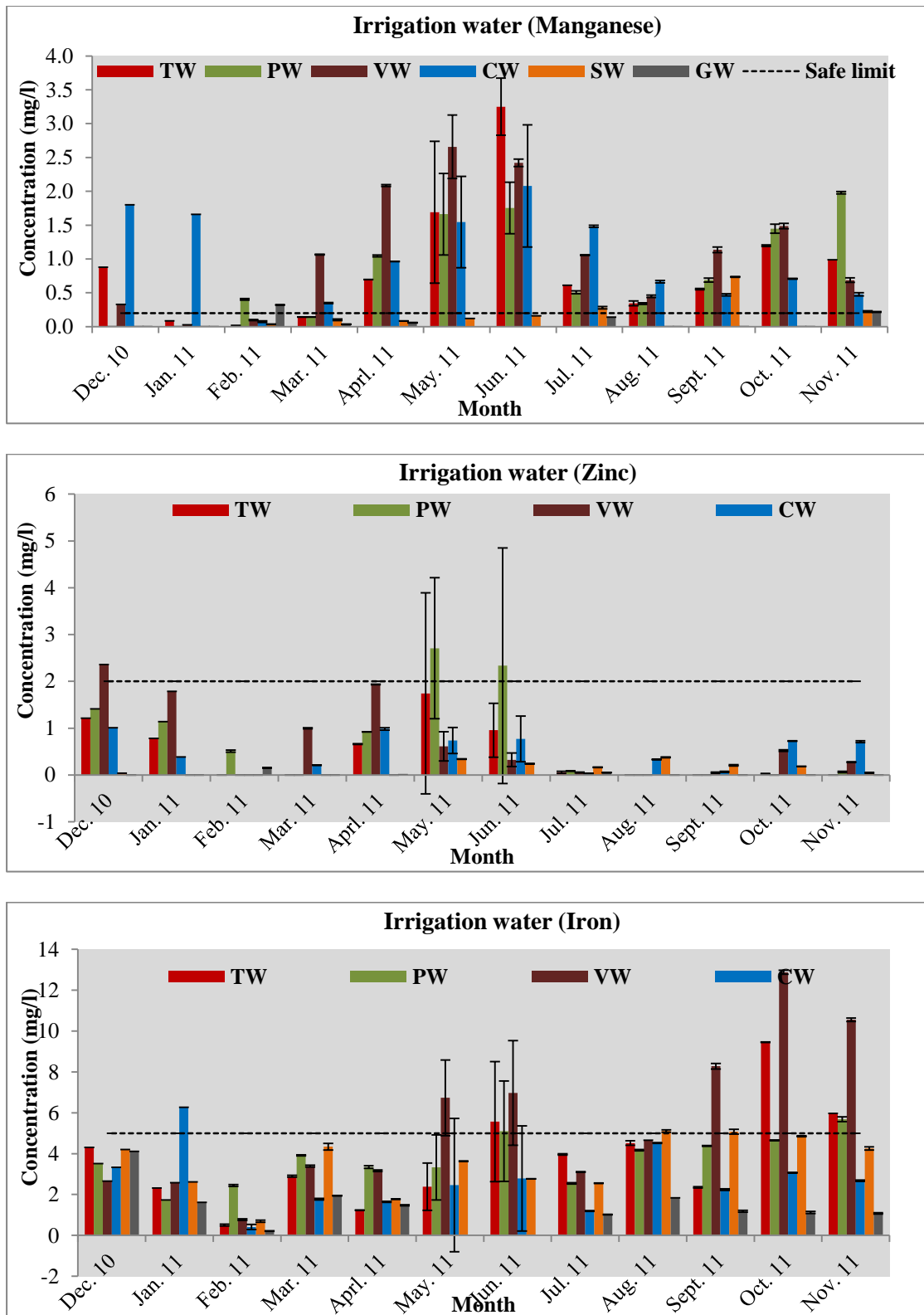


Figure 4.8: Monthly variation in (a) Mn, (b) Zn and (c) Fe concentration in different irrigation water

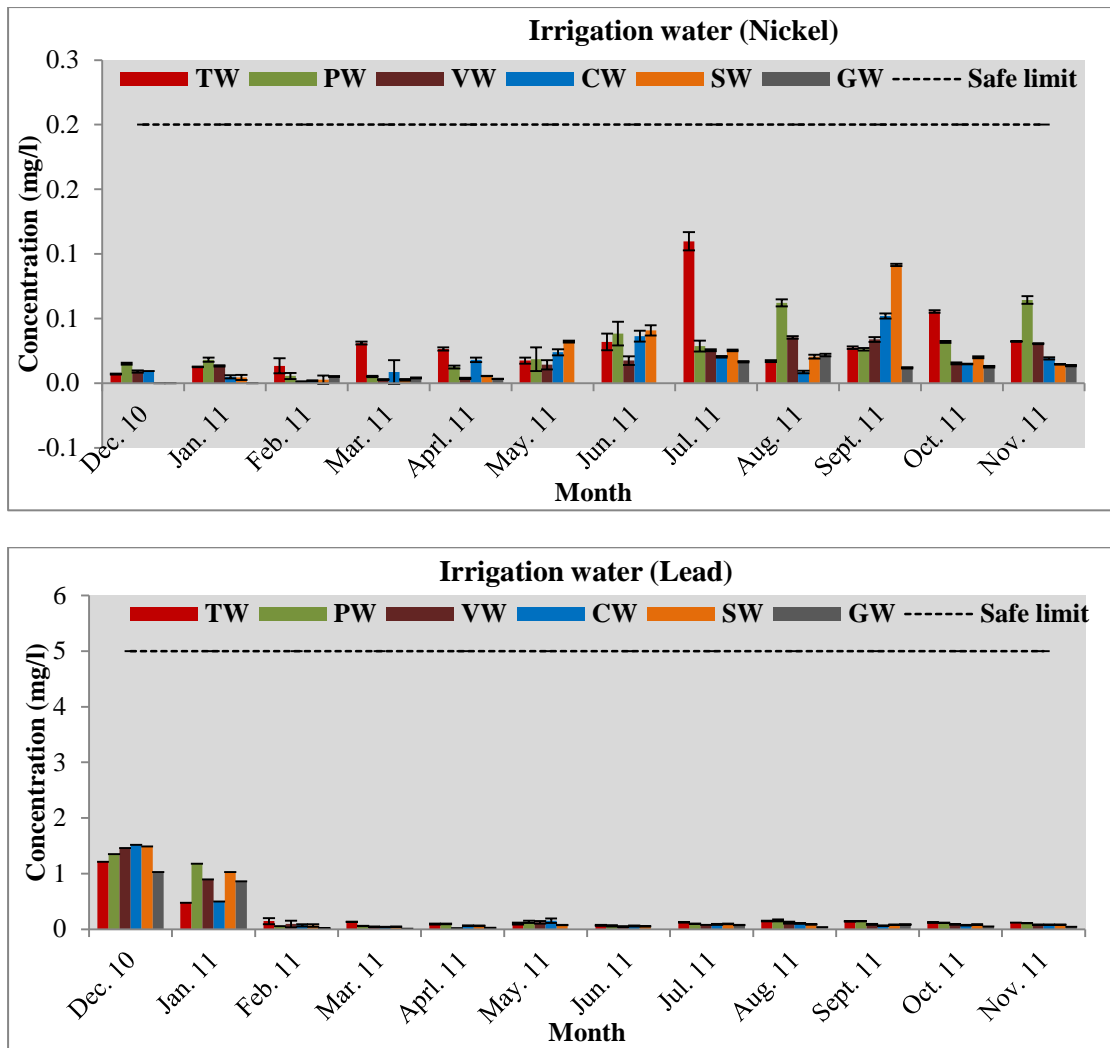


Figure 4.9: Monthly variation in (a) Ni and (b) Pb concentration in different irrigation water

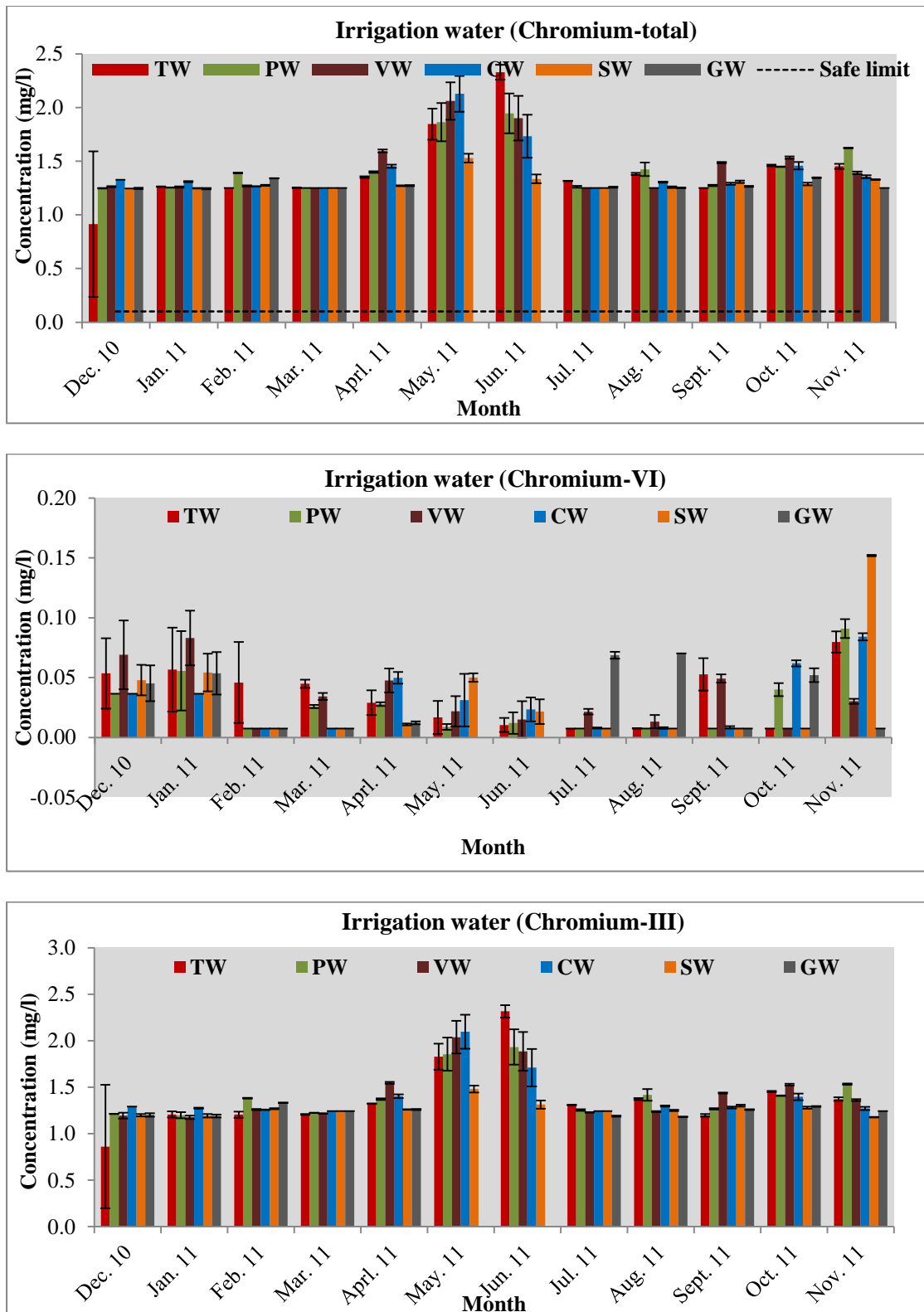


Figure 4.10: Monthly variation in (a) Cr-total, (b) Cr-VI and (c) Cr-III concentration in different irrigation water

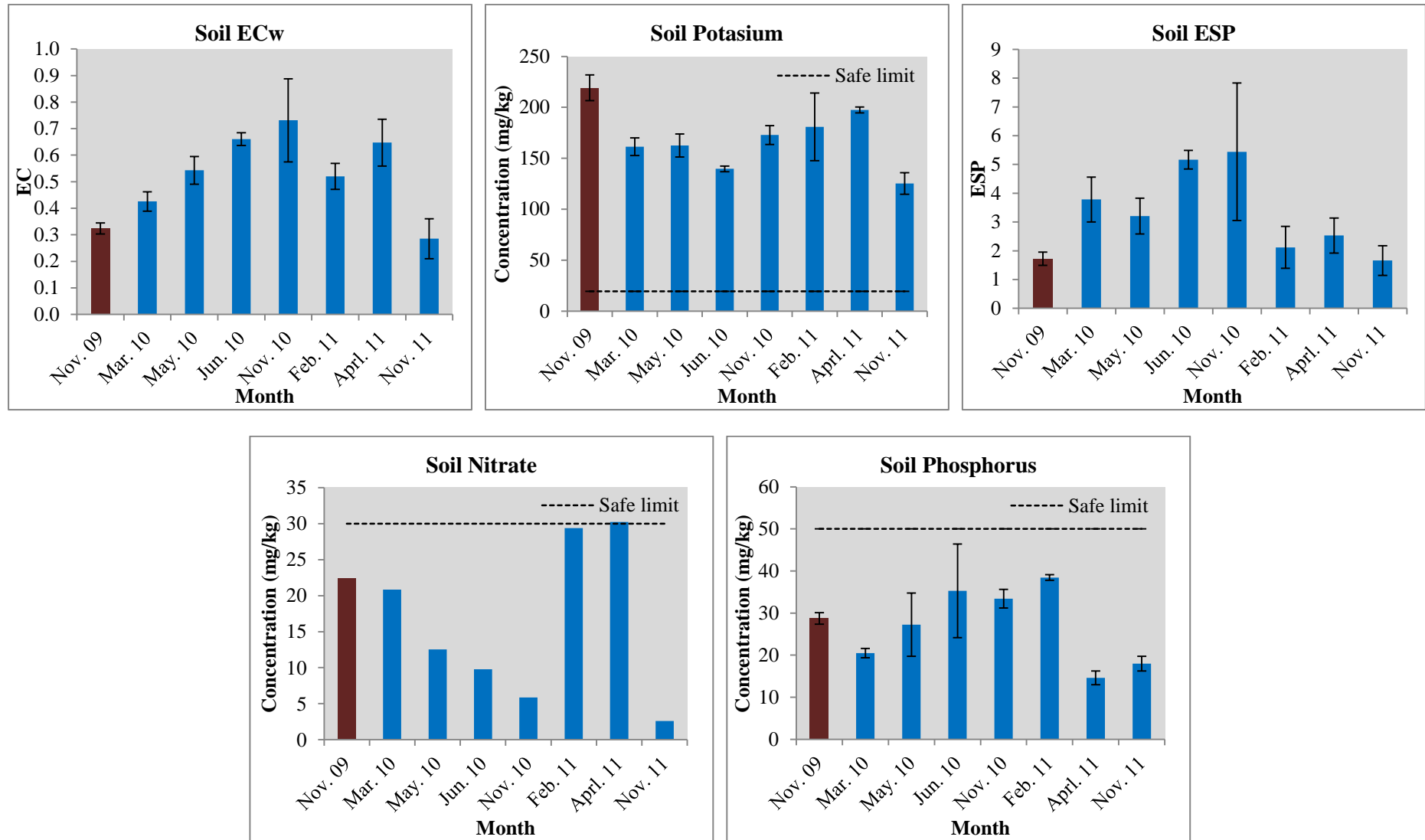


Figure 4.11: Variation in soil (a) ECw, (b) ESP value, (c) Potassium, (d) Nitrate and (e) Phosphate concentration

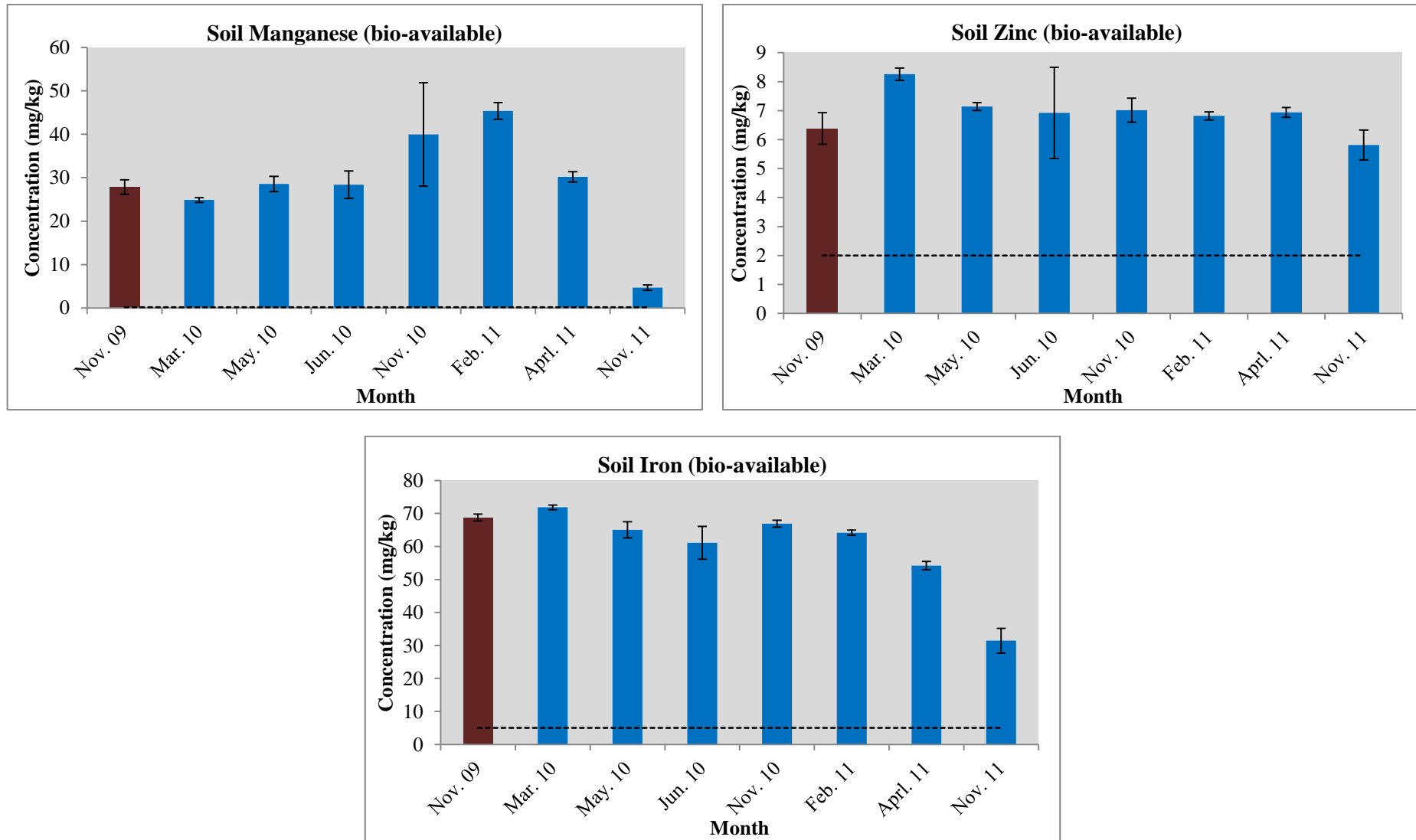


Figure 4.12: Variation in soil bioavailable metal (a) Manganese, (b) Zinc and (c) Iron concentration

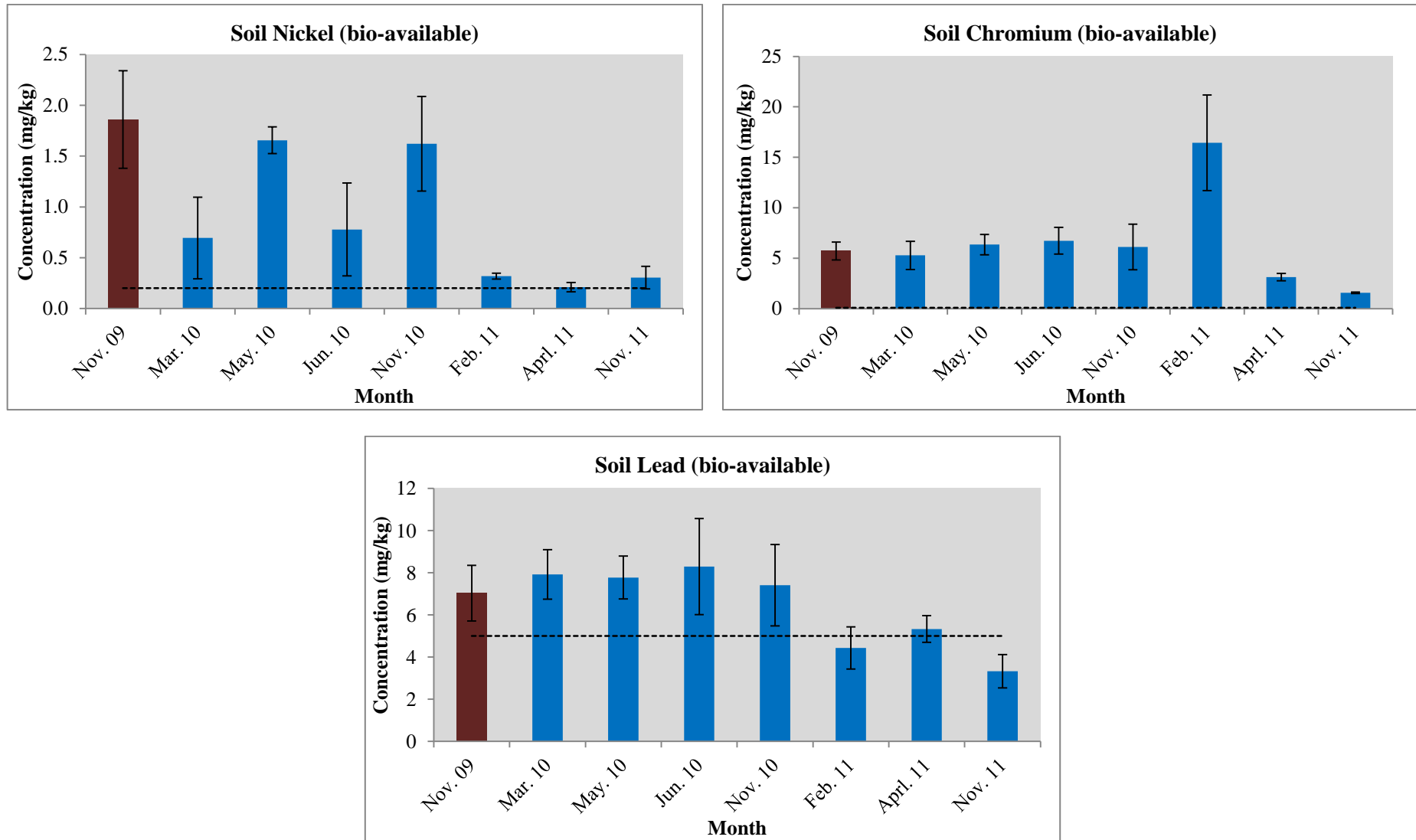


Figure 4.13: Variation in soil bioavailable metal (a) Nickel, (b) Chromium and (c) Lead concentration

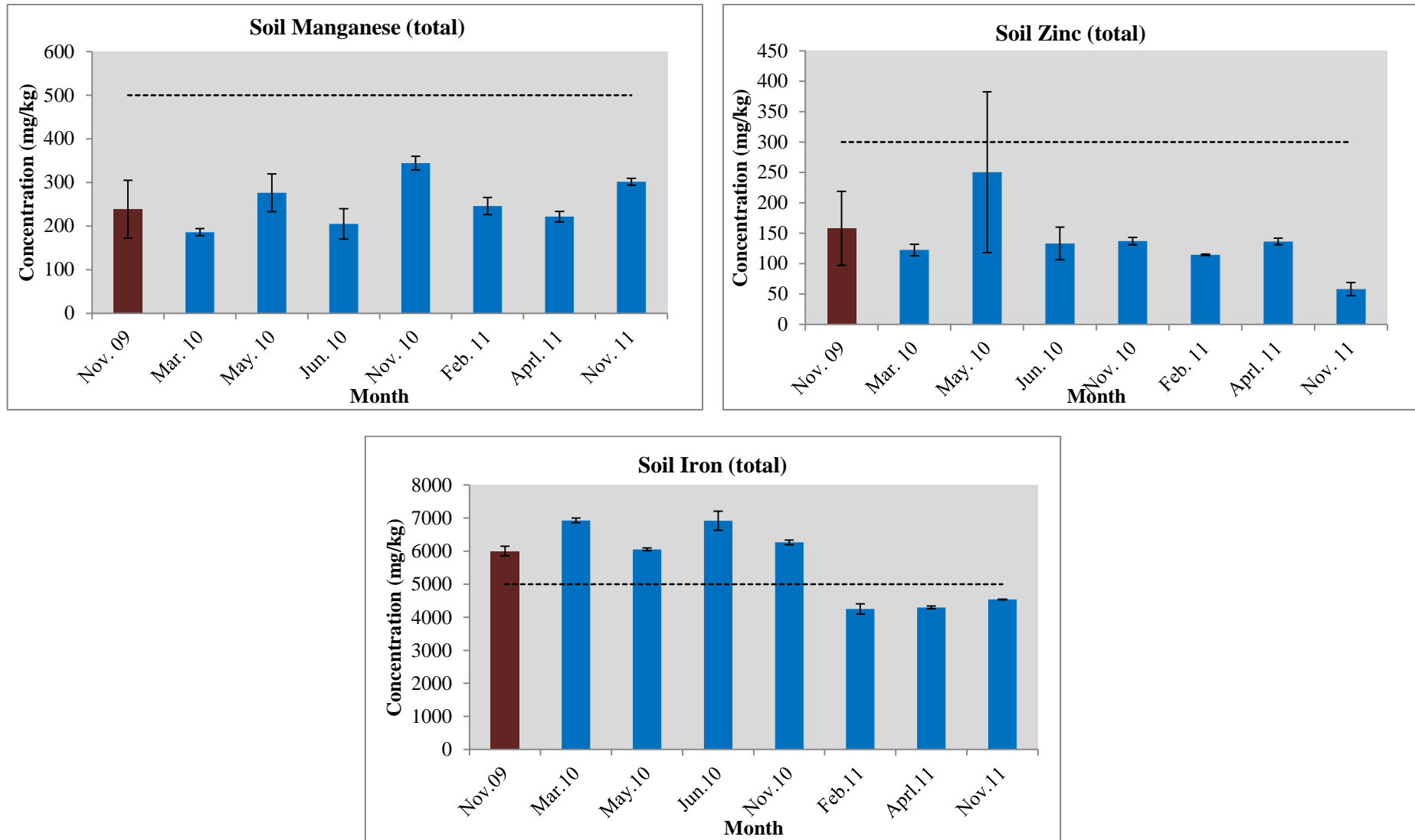


Figure 4.14: Variation in soil total metal (a) Manganese, (b) Zinc and (c) Iron concentration

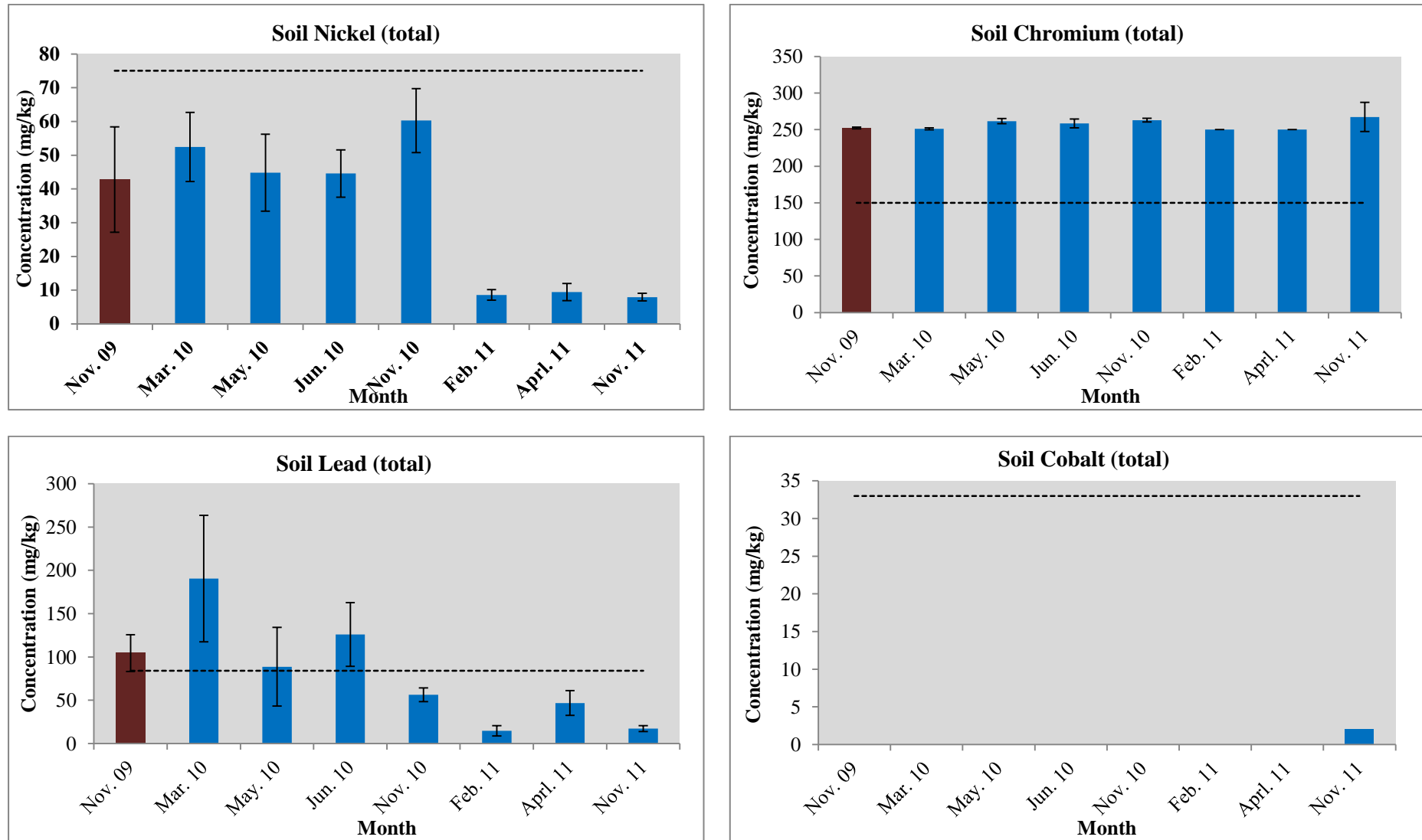


Figure 4.15: Variation in soil total metal (a) Nickel, (b) Chromium and (c) Lead and (d) Cobalt concentration

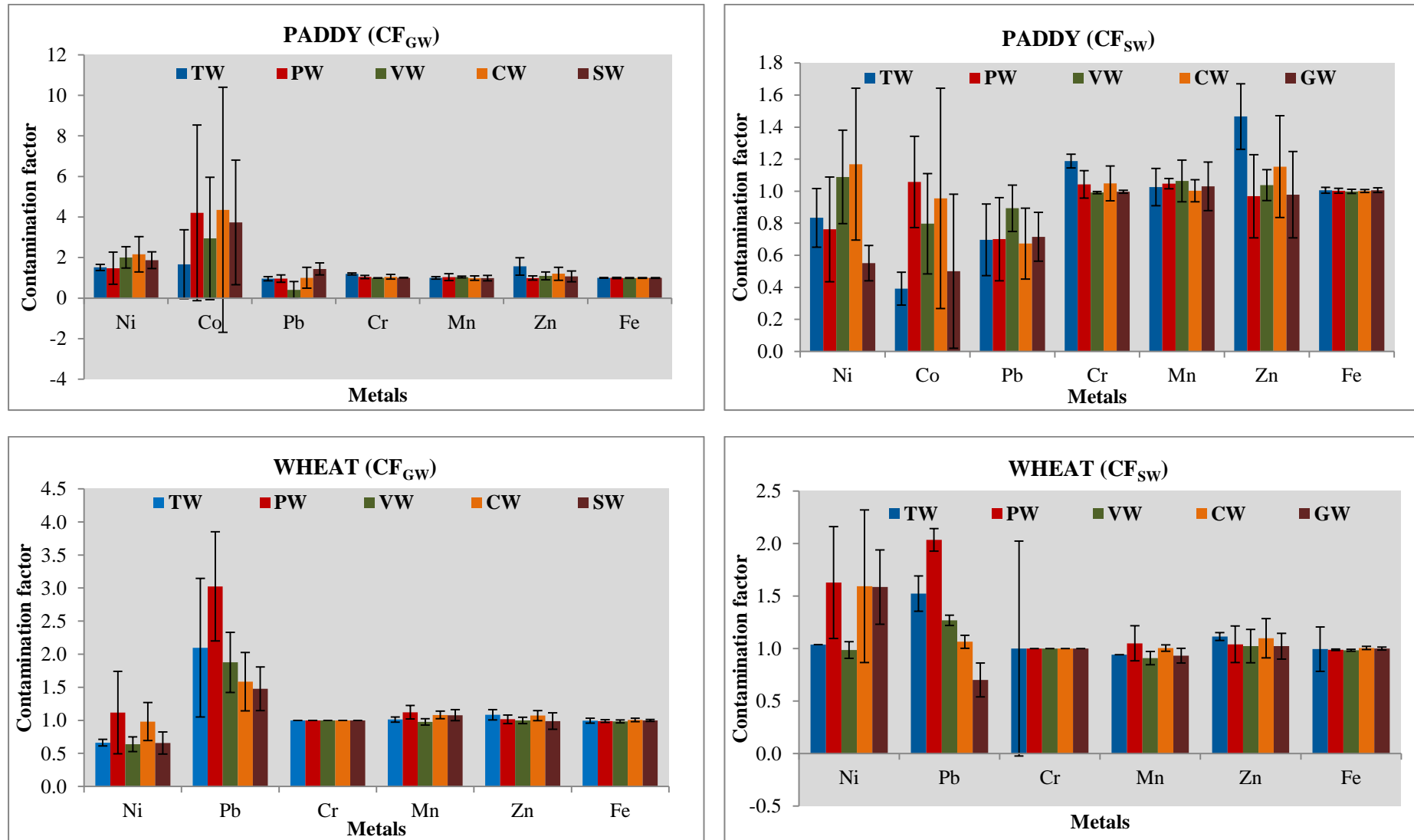


Figure 4.16: Contamination factor of various metals in different irrigation plots in Paddy (a) & (b) and Wheat season (c) & (d) with respect to ground water ( $CF_{GW}$ ) and sewage water ( $CF_{SW}$ ), respectively.

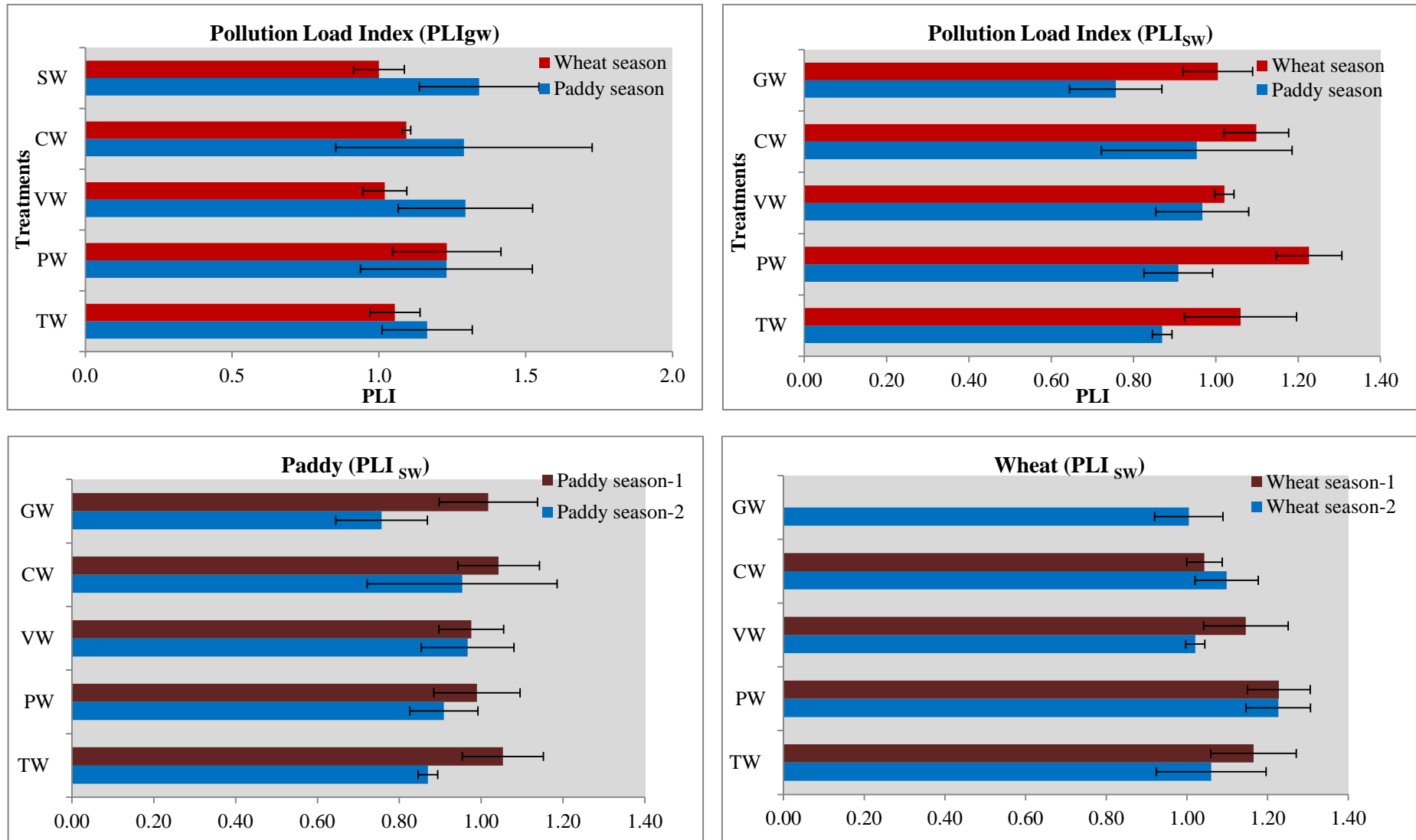


Figure 4.17: Pollution load index of different irrigation plots in Wheat (Rabi) and Paddy (Kharif) season (a) Combined Wheat and Paddy PLI<sub>SW</sub>, (b) Combined Wheat and Paddy PLI<sub>GW</sub> (c) Comparative PLI<sub>SW</sub> of Paddy and (d) Comparative PLI<sub>SW</sub> of Wheat with respect to previous year

## RESEARCH PAPER-II

### To assess impact of wetland treated sewage water applications on the agricultural produce from sewage plot site

---

#### Abstract

The impact of the untreated and treated sewage waters on the health and quality of the Wheat and Paddy crops was estimated in terms of plant/ seed parameters, individual metal translocation pattern and food grain metal sequestration threat. The positive impact of water treatment could be best expressed in terms of the test weight and 100 seed weight of the Paddy crop, as it was found to be significantly lower for the wastewater irrigated crop. Further, though total number of tillers and length of panicle were not significantly different for the treated and the untreated sewage waters, yet total number of unproductive tillers and the unfilled seeds per panicle were significantly higher in the sewage water irrigated paddy crop. These differences were not very evident in the relatively low water demanding crop i.e. wheat seeds produced from treated/ untreated wastewaters. However, number of termite and fungal infected tillers in both wheat and paddy were observed to be higher in the sewage water irrigated micro-plots. Individual metal translocation patterns in the wheat and paddy crop plants revealed higher food grain metal sequestration threat in wheat. In general, wheat grains produced with treated wastewater discharged from wetlands containing *Phragmites karka* and *Acorus calamus* were observed to be sequestering significantly lower lead and nickel/ cadmium concentrations, respectively than those produced with the untreated sewage waters. Similarly paddy grains produced with treated sewage waters discharged from the wetlands containing *Phragmites karka* and *Acorus calamus*/ unplanted mesocosm seemed to be sequestering significantly lower cadmium and iron, respectively.

#### 5.1 Introduction

Water resources scarcity, accessibility, and environmental degradation are the major challenges facing most of the countries including India. An increasing number of countries are now approaching full utilization of their surface and ground freshwater resources. Most of the economically viable development of these resources has been already implemented. In this context treated and re-used sewage water is becoming a common source for additional water in water scarce regions.

Agricultural re-use of wastewater closes the loop between water demand and wastewater disposal since agriculture is the largest user of water (nearly 70% and >90% in some countries) (Gleick, 2000; FAO, 2008). At present, at least one-tenth of the world's population consumes crops irrigated with untreated or poorly treated wastewater, mostly in developing countries in Africa, Asia and Latin America (Smith & Nasr, 1992).

Sewage water contains a lot of plant nutrients whose agricultural re-use helps in nutrient recycling and soil organic matter and fertility increase. However, its unplanned and improper re-use may also lead to excessive build-up of inorganic ions, heavy metals and pathogenic microbes in soil. For instance irrigation waters containing large amounts of sodium, chloride or boron may cause plant/ leaf injury. Excessive necrosis (dead tissue) is often accompanied by early leaf drop or defoliation. The typical visible symptom of boron toxicity is leaf burn chlorotic and/or necrotic patches, often at the margins and tips of older leaves (Bergmann, 1992; Eaton, 1944). Together with such in-organic ions, plants absorb numerous elements from soil. Some of the absorbed elements are referred to as essentials because they are required for plants to complete their life cycle (Arnon and Stout, 1939). Certain essential transition elements such as iron, manganese, molybdenum, copper, zinc, and nickel are known as micronutrients because they are required by plants in minute quantities (Arnon and Stout, 1939). However, plants also absorb elements which have no known biological function and are even known to be toxic at lower concentrations. Among these are arsenic, cadmium, chromium, mercury, and lead. When plants are exposed to excessive heavy metals in soil, they exhibit symptoms of phytotoxicity such as inhibition of seed germination, decreased plant height, decreased tillering, reduced root growth, decreased shoot growth, leaf chlorosis and necrosis, decreased chlorophyll metabolism, and lower fruit and grain yield. Such exposure may also sometimes lead to plant death (Rahman *et al.*, 2007).

Several studies have shown that important soil factors affecting crop metal concentration are soil texture, pH, specific metals concentration, organic matter, and complexing ligands (McLaughlin *et al.*, 1997; Jansson and Oborn, 2000). The metal concentration in crops tends to be the highest in soils with low pH, low organic matter content, high soil metal concentration. Generally a metal will not be absorbed by the plants unless it first reaches a threshold concentration in the soil. Metals are bound to soil with pH above 6.5 and high organic matter content. At pH level below this

organic matter is consumed and metals become mobile and can be absorbed by crops and contaminate water bodies (WHO, 2006). Cadmium, copper, molybdenum, nickel and zinc are frequently present in wastewater and can be mobilized easily and thus absorbed by plants.

Plants have strategies to partition these metals, in different proportions, in different plant parts like root, shoot and grains. Studies on uptake of heavy metals by plants have shown that heavy metals can be transported passively from root to shoot through the xylem vessels (Krijger et al. 1999) but redistribution into the storage organs is largely phloem-dependant and heavy metals have poor mobility in the phloem. Generally, toxic metal accumulation in various plant tissues were reported in the order roots > leaves > stems > inflorescences > seeds. However, it differ from crop to crop and nature of metals ions. Generally vegetables having shorter life period, faster growth, larger leaf area and thus larger transpiration rates area associated with more water and nutrient absorption than cereals and thus show higher soil metal absorption/ accumulation efficiency than cereals. However among different vegetables leafy vegetables accumulate more metals than root crops (Nielsen et al. 1998; Puschenreiter et al. 2005). High concentration of metals in the agricultural produce may further affect consumer health and thus whole food chain.

Environmental and human health risks due to wastewater agriculture/ use can be minimized by suitable treatment of wastewater before use, careful handling, wise application and proper awareness. Non-conventional wastewater treatment methods include the use of low-cost systems such as on-farm ponds, sedimentation traps, bio sand-filters and constructed wetlands. Of these, wetland systems have shown promise in achieving large reductions in nutrient/ toxic levels and in coliform bacteria. However, there are very few studies to account for an overall impact of the wetland treated wastewaters on the quality of agricultural produce and thus human health.

With this in background, the present investigation was primarily aimed at assessing impact of wetland treated sewage water applications on the agricultural produce from a historically sewage water irrigated site at Indian Agricultural Research Institute.

## **5.2 Material and methods**

A pilot scale vertical sub-surface flow (VSSF) constructed wetland system was developed and sited, near a domestic sewage water drain, on a 350 m<sup>2</sup> land

area (28°38'21.3" N and 77°08'56.5" E) within the Seed Production Unit (SPU) farm of the Indian Agricultural Research Institute (IARI). Sewage waters generated from the IARI micro-watershed (containing moderate organic load, lab chemical compounds and heavy metals) were passed through the aforementioned treatment wetlands with 4 replicates of *Typha latifolia* (Cattail), *Phragmites karka* (Reed), *Acorus calamus* (Vacha) and six replicates of unplanted mesocosms to generate 4 types of treated wastewaters such as *Typha latifolia* (Cattail) treated water (TW), *Phragmites karka* (Reed) treated water (PW), *Acorus calamus* (Vacha) treated water (VW), Control (No vegetation) treated water (CW). These 4 types of treated waters (discharged from the aforementioned treatment wetlands) along with the 2-*business-as-usual* irrigation strategies viz. the sewage (SW) and ground waters (GW) irrigations were then used for irrigating paddy (Pusa Basmati-1121) and wheat (Variety HD-2894) crops, as per the standard agronomic practices, in the adjacent 18 - micro-plots (each of 1.8m × 1.5m = 2.7 m<sup>2</sup> size), with sandy loam type soil and pre-contaminated history of sewage water application. The aforementioned six irrigation treatments viz. *Typha latifolia* (Cattail) treated water (TW), *Phragmites karka* (Reed) treated water (PW), *Acorus calamus* (Vacha) treated water (VW), Control (No vegetation) treated water (CW), Raw Sewage water (SW) and Ground water (GW) irrigation micro-plots were arranged in randomized block design (RBD); each with three replications.

For analysing the impact of water treatments on the crop plants and their produce, plant samples were collected. In case of wheat, four plants each of three height groups viz. long (Main), medium (Mid) & short (Low) of total twelve healthy plants and in case of paddy, three tillers of three height groups viz. long (Main), medium (Mid) & short (Lower) of total five healthy plants per each micro-plot were randomly tagged, just after grain filling stage and used for analysis. These tagged plants having intact root were sampled with khurpi just before harvesting of the rest of the plants. Each micro-plot plant sample was bundled and immediately transferred to laboratory and air dried. On the air dried plant samples different plant parameters (as detailed in section 3.3.2.3) were analysed and their separated root, shoot and grain parts were packed in brown envelopes. Root and shoot parts were oven dried (at 55°C) and ground (individually) along with grains through a milling machine and subjected to micro-nutrient (viz. Zn, Cu, Fe, Mn) and trace metal (viz. Cd, Cr, Co, Ni, Pb) analysis as per the procedure detailed in section (3.3.2.3). All samples were

analysed in triplicate; with due quality control ensured through careful standardization, procedural blank measurements and duplicate samples in the Hydro-informatics and Wetland bio-geochemistry laboratories of the Division of Environmental Sciences, IARI. Seed germination tests were performed in the seed testing laboratory of Seed Science Technology Division of IARI.

Extent of pollutant transfer from soil to plant and its transfer/ translocation to different plant parts was evaluated through bio-concentration (BCF, Barman et al., 2000) and translocation factors (TF, Cui et al., 2004). Their computational procedures are detailed in section 3.4). Enrichment factors (EF, Buat-Menard & Chesselet, 1979) for estimating translocation of each heavy metal from the contaminated soil to the plant tissue, w.r.t. the good quality water irrigated soils, was also computed as per the procedures detailed in section 3.4). Enrichment factor of heavy metals depends upon bioavailability of metals, which in turn depends upon its concentration in soil, their chemical forms, difference in uptake capability and growth rate of different plant species (Tinker, 1981). Higher values of enrichment factor (EF) suggest poor retention of metals in soil and/or their more translocation in plants.

### **5.3 Results and Discussions**

The impact of the untreated and treated sewage waters on the health and quality of the Wheat and Paddy crops was estimated in terms of plant/ seed parameters, individual metal translocation pattern and food grain metal sequestration threat.

#### *Plant and seed parameters*

The analysis showed that the biological yield of Wheat (Figure 5.1b) and Paddy (Figure 5.1a) crops irrigated with the treated and the untreated sewage waters were not significantly different. In general, the economic yield and harvest index of the Wheat (Figure 5.1d and 5.4d) and Paddy (Figure 5.1c and 5.4c) crops, irrigated with treated and untreated sewage waters, were also not significantly different. However, in case of the Paddy crop, both economic yield ( $2.03 \pm 0.05$  kg/plot) and harvest index ( $0.39 \pm 0.04$ ) were found to be significantly higher with the treated irrigation waters discharged from the unplanted (CW) wetlands. Paddy crop is generally associated with larger amounts of irrigation water applications and thus pollutant exposure. Long term pollutant mass reduction efficiency of varied wetland systems (Kaur et al., 2011) indicated that over two years the unplanted wetlands,

associated with higher hydraulic retention time (HRT ~ 2.5 days), could yield about 10 to 20% higher pollutant mass reduction than the rest of the (so far not harvested) planted wetland systems (associated with lower HRTs i.e. ~ 14 to 16 hrs.). Thus, as expected, it appears that lower nutrient and metal loads in the irrigation waters treated and discharged by the unplanted mesocosms/ wetlands translated into higher economic yield and harvest index in the paddy crop. However, these differences were not significant in the wheat crop due to the incorporation of far lower (just 1/3<sup>rd</sup>) irrigation volumes and thus exposure to pollutants in the plant root zone.

The positive impact of water treatment could be best expressed in terms of the test weight/ 100 seed weight of the Paddy crop (Figure 5.3d and 5.3e) as it was found to be significantly lower for the wastewater (SW) irrigated crop. Even the groundwater (GW) irrigated crop could not yield as high test/ 100 seed weight as the treated wastewater irrigated crop.

Though, total number of tillers and length of the panicle (both economic portion and panicle exertion length) were not significantly different for the treated and the untreated sewage waters, yet total number of unproductive tillers (Figure 5.3c) and the unfilled seeds per panicle (Figure 5.3a) were significantly higher in the sewage water irrigated paddy crop.

Vigour Index-I and II were also computed for the paddy and the wheat seeds. The analysis clearly depicted that sewage water produced paddy seeds were associated with significantly lower vigour than those produced through the treated wastewaters. However these differences were not very evident in the relatively low water demanding crop i.e. wheat seeds produced from treated/ untreated wastewaters.

Even total number of fungal infected tillers was significantly lower in the treated sewage water irrigated crop (Figure 5.4a). Amongst the treated sewage water irrigated micro-plots, those receiving treated wastewaters from the unplanted mesocosms/ wetland (CW) were observed to be associated with the lowest fungal attack. This was also observed to be the case for the termite infected Wheat tillers (Figure 5.4b), which were observed to be more infected in the sewage water irrigated plots.

#### *Metal translocation pattern and food grain metal sequestration*

Individual metal bio-concentration (Table 5.1 and 5.2; Figure 5.14, 5.15, 5.16 and 5.17) and translocation pattern (Table 5.3 and 5.4; Figure 5.18, 5.19, 5.20 and

5.21) in the Paddy and Wheat crops were also worked out. The analysis clearly revealed that unlike paddy crop, wherein metal translocation pattern was roots >> shoots > grains; in wheat crop there seemed to be a proportionately higher translocation of metals (in general) to the shoots and the grains. For instance though of the total soil metal load present, the percent soil metal bio-available pool concentrations for the Fe (1%), Zn (5-7%), and Pb (15-16%) were the same for both wheat and paddy soils, yet only about 1.7% (Figure 5.16c), 26% (Figure 5.16b), and 61% (Figure 5.17b), respectively could be translocated to the paddy grains while 11% (Figure 5.14c), 166% (Figure 5.14b), and 83% (Figure 5.15b), respectively could be translocated to the wheat grains. Similarly in the case of Mn and Cr, the average percent metal translocation to paddy grains was 18.9% (Figure 5.20a) and 10% (Figure 5.21d), respectively while that in wheat grains was 203% (Figure 5.18a) and 23% (Figure 5.19d), respectively. However, it was observed that this could also be to some extent due to higher Mn and Cr bio-available pool concentrations in the wheat soils (Mn: 14%; Cr: 4%) as compared to the paddy soils (Mn: 7%; Cr: 1%). Thus, in general, wheat grains seemed to be at more risk to food grain metal contamination than the paddy grains.

Table 5.1: Bio-concentration factor of various metals in wheat

<b>METAL</b>		<b>TW</b>	<b>PW</b>	<b>VW</b>	<b>CW</b>	<b>SW</b>	<b>GW</b>
<b>Ni</b>	<b>Root</b>	52.55 ± 8.13	46.90 ± 3.91	65.72 ± 4.20	80.09 ± 14.66	70.87 ± 7.17	134.07 ± 14.03
	<b>Shoot</b>	64.42 ± 14.46	52.93 ± 1.65	81.60 ± 12.34	98.58 ± 11.14	84.22 ± 5.24	168.27 ± 18.83
	<b>Grain</b>	54.20 ± 4.91	38.02 ± 3.12	52.60 ± 7.64	66.56 ± 8.20	57.10 ± 1.28	100.79 ± 3.86
<b>Cr</b>	<b>Root</b>	71.06 ± 18.06	88.20 ± 10.22	87.32 ± 2.78	75.93 ± 0.10	86.15 ± 3.76	74.05 ± 1.11
	<b>Shoot</b>	71.24 ± 17.76	88.20 ± 10.22	86.92 ± 2.63	75.93 ± 0.10	86.70 ± 3.82	74.05 ± 1.11
	<b>Grain</b>	18.03 ± 6.57	18.29 ± 1.82	17.38 ± 0.53	18.00 ± 3.28	21.85 ± 2.44	17.69 ± 2.49
<b>Pb</b>	<b>Root</b>	5.67 ± 1.97	3.39 ± 0.98	2.45 ± 0.46	3.15 ± 0.56	2.52 ± 0.81	2.59 ± 0.28
	<b>Shoot</b>	4.75 ± 0.79	2.33 ± 1.08	1.87 ± 0.27	3.05 ± 0.78	1.90 ± 0.40	1.78 ± 0.10
	<b>Grain</b>	3.24 ± 1.00	1.22 ± 0.52	1.77 ± 1.18	3.15 ± 0.95	2.75 ± 1.37	2.63 ± 0.06
<b>Mn</b>	<b>Root</b>	1.60 ± 0.67	1.00 ± 0.26	1.05 ± 0.24	1.59 ± 0.35	0.87 ± 0.32	1.14 ± 0.31
	<b>Shoot</b>	0.97 ± 0.11	1.02 ± 0.17	1.07 ± 0.30	1.17 ± 0.26	0.87 ± 0.25	0.87 ± 0.24
	<b>Grain</b>	2.14 ± 0.14	2.22 ± 1.33	2.23 ± 1.43	3.75 ± 1.29	2.09 ± 1.29	1.20 ± 0.29
<b>Zn</b>	<b>Root</b>	5.39 ± 1.44	4.58 ± 0.95	6.25 ± 0.33	4.81 ± 1.51	4.26 ± 1.52	4.92 ± 1.43
	<b>Shoot</b>	3.97 ± 0.37	3.76 ± 0.43	3.68 ± 0.33	3.33 ± 0.81	3.54 ± 0.21	3.04 ± 0.70
	<b>Grain</b>	7.88 ± 0.71	8.52 ± 2.35	8.50 ± 0.41	7.47 ± 1.06	7.65 ± 1.09	7.88 ± 0.74
<b>Fe</b>	<b>Root</b>	29.63 ± 14.14	24.81 ± 6.33	23.02 ± 6.30	27.01 ± 10.07	23.35 ± 11.38	27.85 ± 7.87
	<b>Shoot</b>	12.62 ± 0.31	13.67 ± 4.88	13.09 ± 3.58	13.40 ± 1.45	11.82 ± 0.33	14.29 ± 6.52
	<b>Grain</b>	4.35 ± 2.84	2.44 ± 1.92	4.01 ± 3.38	1.67 ± 0.91	1.95 ± 0.70	0.82 ± 0.45

Table 5.2: Bio-concentration factor of various metals in paddy

METAL		TW	PW	VW	CW	SW	GW
Ni	Root	35.66 ± 15.45	33.70 ± 2.29	79.58 ± 34.89	36.78 ± 29.77	34.81 ± 13.26	51.47 ± 14.95
	Shoot	14.34 ± 1.54	13.64 ± 5.05	19.83 ± 4.18	9.73 ± 2.88	18.44 ± 5.43	16.37 ± 4.20
	Grain	18.49 ± 1.78	12.42 ± 2.20	39.77 ± 14.66	8.98 ± 0.63	12.42 ± 2.84	15.93 ± 2.78
Cr	Root	164.36 ± 33.05	165.92 ± 15.49	165.44 ± 8.47	163.37 ± 18.66	151.72 ± 8.22	167.62 ± 3.15
	Shoot	18.45 ± 3.03	16.52 ± 1.58	16.85 ± 1.30	17.19 ± 2.47	15.36 ± 0.53	16.76 ± 0.31
	Grain	16.36 ± 3.33	16.38 ± 1.56	16.54 ± 0.85	16.34 ± 1.87	14.80 ± 0.76	16.76 ± 0.31
Pb	Root	5.32 ± 0.99	8.34 ± 1.74	0.13 ± 0.13	7.18 ± 0.25	4.59 ± 0.59	4.22 ± 0.34
	Shoot	1.79 ± 0.67	3.76 ± 0.40	0.07 ± 0.07	2.26 ± 0.15	3.95 ± 0.18	3.31 ± 0.25
	Grain	4.15 ± 0.49	1.71 ± 0.77	1.12 ± 1.12	3.11 ± 0.94	2.29 ± 1.00	6.43 ± 0.60
Mn	Root	24.62 ± 7.54	19.71 ± 5.78	15.79 ± 6.91	9.13 ± 2.17	10.87 ± 4.34	5.45 ± 1.57
	Shoot	8.88 ± 2.48	7.07 ± 7.02	4.74 ± 2.11	5.80 ± 2.44	4.44 ± 1.59	1.70 ± 1.56
	Grain	2.68 ± 0.85	3.30 ± 0.86	1.88 ± 1.05	2.46 ± 0.56	2.35 ± 0.17	1.24 ± 0.96
Zn	Root	9.50 ± 2.56	11.78 ± 2.97	11.38 ± 2.62	11.65 ± 5.58	13.68 ± 3.81	8.54 ± 2.17
	Shoot	5.80 ± 2.10	7.51 ± 0.47	6.42 ± 0.70	6.64 ± 2.76	7.83 ± 2.26	7.08 ± 2.05
	Grain	3.32 ± 1.39	3.26 ± 1.23	3.56 ± 2.20	2.00 ± 0.97	3.11 ± 0.17	1.90 ± 1.33
Fe	Root	127.77 ± 29.22	132.07 ± 12.74	128.89 ± 20.11	154.51 ± 7.37	131.54 ± 11.61	135.36 ± 23.23
	Shoot	10.62 ± 2.46	10.64 ± 2.39	10.72 ± 2.06	14.28 ± 0.75	13.37 ± 2.64	12.97 ± 3.23
	Grain	3.46 ± 1.29	1.64 ± 0.84	1.26 ± 1.68	1.34 ± 2.26	2.69 ± 1.61	2.98 ± 1.68
Co	Root	887.03 ± 513.15	288.30 ± 62.37	501.46 ± 730.75	80.04 ± 39.97	995.43 ± 363.64	932.05 ± 859.21
	Shoot	48.49 ± 45.56	9.13 ± 15.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.80 ± 37.84
	Grain	25.16 ± 4.05	0.32 ± 0.55	18.56 ± 19.02	0.00 ± 0.00	4.22 ± 7.30	5.42 ± 9.38

Table 5.3: Translocation Factor (TF) of various metals in wheat

<b>METAL</b>		<b>TW</b>	<b>PW</b>	<b>VW</b>	<b>CW</b>	<b>SW</b>	<b>GW</b>
<b>Ni</b>	<b>Shoot</b>	1.24 ± 0.31	1.13 ± 0.06	1.24 ± 0.12	1.24 ± 0.13	1.20 ± 0.14	1.27 ± 0.25
	<b>Grain</b>	1.04 ± 0.09	0.81 ± 0.03	0.80 ± 0.09	0.84 ± 0.10	0.81 ± 0.07	0.76 ± 0.05
<b>Cr</b>	<b>Shoot</b>	1.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.01	1.00 ± 0.00	1.01 ± 0.01	1.00 ± 0.00
	<b>Grain</b>	0.25 ± 0.03	0.21 ± 0.01	0.20 ± 0.00	0.24 ± 0.04	0.25 ± 0.04	0.24 ± 0.03
<b>Pb</b>	<b>Shoot</b>	0.87 ± 0.16	0.70 ± 0.24	0.79 ± 0.20	0.96 ± 0.11	0.80 ± 0.24	0.69 ± 0.06
	<b>Grain</b>	0.58 ± 0.02	0.42 ± 0.33	0.69 ± 0.41	1.04 ± 0.43	1.20 ± 0.83	1.02 ± 0.08
<b>Mn</b>	<b>Shoot</b>	0.65 ± 0.17	1.12 ± 0.53	1.05 ± 0.30	0.76 ± 0.25	1.20 ± 0.83	0.77 ± 0.20
	<b>Grain</b>	1.46 ± 0.44	2.34 ± 1.32	2.47 ± 2.21	2.33 ± 0.56	2.47 ± 1.12	1.08 ± 0.30
<b>Zn</b>	<b>Shoot</b>	0.77 ± 0.20	0.84 ± 0.17	0.59 ± 0.08	0.70 ± 0.06	0.92 ± 0.39	0.65 ± 0.25
	<b>Grain</b>	1.51 ± 0.31	1.88 ± 0.45	1.36 ± 0.07	1.68 ± 0.59	1.90 ± 0.44	1.66 ± 0.30
<b>Fe</b>	<b>Shoot</b>	0.48 ± 0.19	0.61 ± 0.38	0.59 ± 0.21	0.54 ± 0.17	0.61 ± 0.34	0.50 ± 0.13
	<b>Grain</b>	0.19 ± 0.18	0.11 ± 0.12	0.18 ± 0.16	0.06 ± 0.04	0.11 ± 0.09	0.03 ± 0.01
<b>Co</b>	<b>Shoot</b>	2.81 ± 0.25	2.35 ± 0.88	2.85 ± 0.12	2.95 ± 0.17	2.79 ± 0.18	2.89 ± 0.54
	<b>Grain</b>	1.04 ± 0.02	0.85 ± 0.17	0.96 ± 0.04	0.97 ± 0.19	0.93 ± 0.03	0.88 ± 0.07

Table 5.4: Translocation Factor (TF) of various metals in paddy

METAL		TW	PW	VW	CW	SW	GW
Ni	Shoot	0.46 ± 0.21	0.41 ± 0.17	0.28 ± 0.09	0.37 ± 0.20	0.54 ± 0.04	0.33 ± 0.11
	Grain	0.57 ± 0.18	0.37 ± 0.09	0.56 ± 0.23	0.43 ± 39	0.37 ± 0.06	0.33 ± 0.13
Cr	Shoot	0.11 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
	Grain	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Pb	Shoot	0.33 ± 0.06	0.46 ± 0.08	0.03 ± 0.03	0.32 ± 0.03	0.87 ± 0.09	0.79 ± 0.11
	Grain	0.79 ± 0.06	0.21 ± 0.10	0.21 ± 0.21	0.43 ± 0.12	0.49 ± 0.019	1.53 ± 0.21
Mn	Shoot	0.364 ± 0.061	0.400 ± 0.426	0.308 ± 0.125	0.690 ± 0.373	0.491 ± 0.366	0.279 ± 0.181
	Grain	0.111 ± 0.030	0.182 ± 0.085	0.120 ± 0.039	0.272 ± 0.057	0.237 ± 0.079	0.210 ± 0.102
Zn	Shoot	0.60 ± 0.09	0.67 ± 0.18	0.58 ± 0.10	0.58 ± 0.08	0.57 ± 0.01	0.83 ± 0.05
	Grain	0.34 ± 0.07	0.30 ± 0.15	0.32 ± 0.18	0.17 ± 0.07	0.24 ± 0.06	0.20 ± 0.12
Fe	Shoot	0.084 ± 0.013	0.081 ± 0.021	0.083 ± 0.008	0.093 ± 0.007	0.101 ± 0.011	0.097 ± 0.024
	Grain	0.030 ± 0.019	0.013 ± 0.007	0.011 ± 0.016	0.008 ± 0.014	0.021 ± 0.013	0.021 ± 0.010
Co	Shoot	0.068 ± 0.088	0.029 ± 0.051	0.000 ± 0.00	0.000 ± 0.00	0.000 ± 0.00	0.038 ± 0.034
	Grain	0.035 ± 0.017	0.001 ± 0.002	0.092 ± 0.135	0.000 ± 0.00	0.006 ± 0.010	0.005 ± 0.009

Further nickel, lead and chromium bio-concentrations in wheat (Figure 5.15a, b, c) and paddy grains (Figure 5.17a, b, d) produced from the sewage waters were about 2-3 times higher than those produced from the treated wastewaters. This was also reflected from the individual metal enrichment factors for the wheat (Table 5.6 and Figure 5.22b) and paddy (Table 5.5 and Figure 5.22a) crops. However, though metal concentrations in the food produced from the micro-plots irrigated with the treated sewage waters were significantly lower than those produced through sewage waters yet these were (2 - 6 times) higher than their maximum allowable levels (MAL).

With respect to the rest of the metals, these differences were not very conspicuous in this short term (one year) study, conducted on the historically sewage water irrigated and thus metal contaminated soils. Thus, the analysis could clearly re-confirm the need for proper treatment and scientific application of the poor quality irrigation waters for maintaining sustainable land / food qualities.

Table 5.5: Enrichment factor (EF) of various metals in paddy grain

<b>SAMPLE</b>	<b>Ni</b>	<b>Pb</b>	<b>Cr</b>	<b>Mn</b>	<b>Zn</b>	<b>Fe</b>
<b>TW</b>	1.19 ± 0.28	0.65 ± 0.09	0.98 ± 0.21	3.10 ± 2.36	5.04 ± 7.06	1.91 ± 2.09
<b>PW</b>	0.80 ± 0.20	0.27 ± 0.15	0.98 ± 0.08	3.80 ± 2.35	4.50 ± 5.98	0.93 ± 1.03
<b>VW</b>	2.47 ± 0.59	0.15 ± 0.15	0.99 ± 0.04	1.71 ± 0.61	3.92 ± 4.42	0.49 ± 0.47
<b>CW</b>	0.58 ± 0.15	0.48 ± 0.11	0.97 ± 0.11	2.56 ± 1.23	1.43 ± 0.82	0.32 ± 0.50
<b>SW</b>	0.82 ± 0.34	0.37 ± 0.18	0.88 ± 0.06	2.68 ± 1.56	3.44 ± 3.88	1.53 ± 1.66

Table 5.6: Enrichment factor (EF) of various metals in wheat grain

<b>SAMPLE</b>	<b>Ni</b>	<b>Pb</b>	<b>Cr</b>	<b>Mn</b>	<b>Zn</b>	<b>Fe</b>
<b>TW</b>	0.54 ± 0.06	1.24 ± 0.40	1.05 ± 0.47	1.84 ± 0.42	1.01 ± 0.16	7.09 ± 6.58
<b>PW</b>	0.38 ± 0.02	0.46 ± 0.20	1.06 ± 0.27	1.74 ± 0.73	1.07 ± 0.20	3.50 ± 3.54
<b>VW</b>	0.52 ± 0.06	0.68 ± 0.46	1.00 ± 0.15	1.81 ± 0.97	1.08 ± 0.07	6.53 ± 7.32
<b>CW</b>	0.66 ± 0.08	1.20 ± 0.38	1.02 ± 0.14	3.06 ± 0.39	0.96 ± 0.23	2.16 ± 1.32
<b>SW</b>	0.57 ± 0.01	1.04 ± 0.51	1.26 ± 0.32	1.68 ± 0.73	0.97 ± 0.10	2.71 ± 1.41

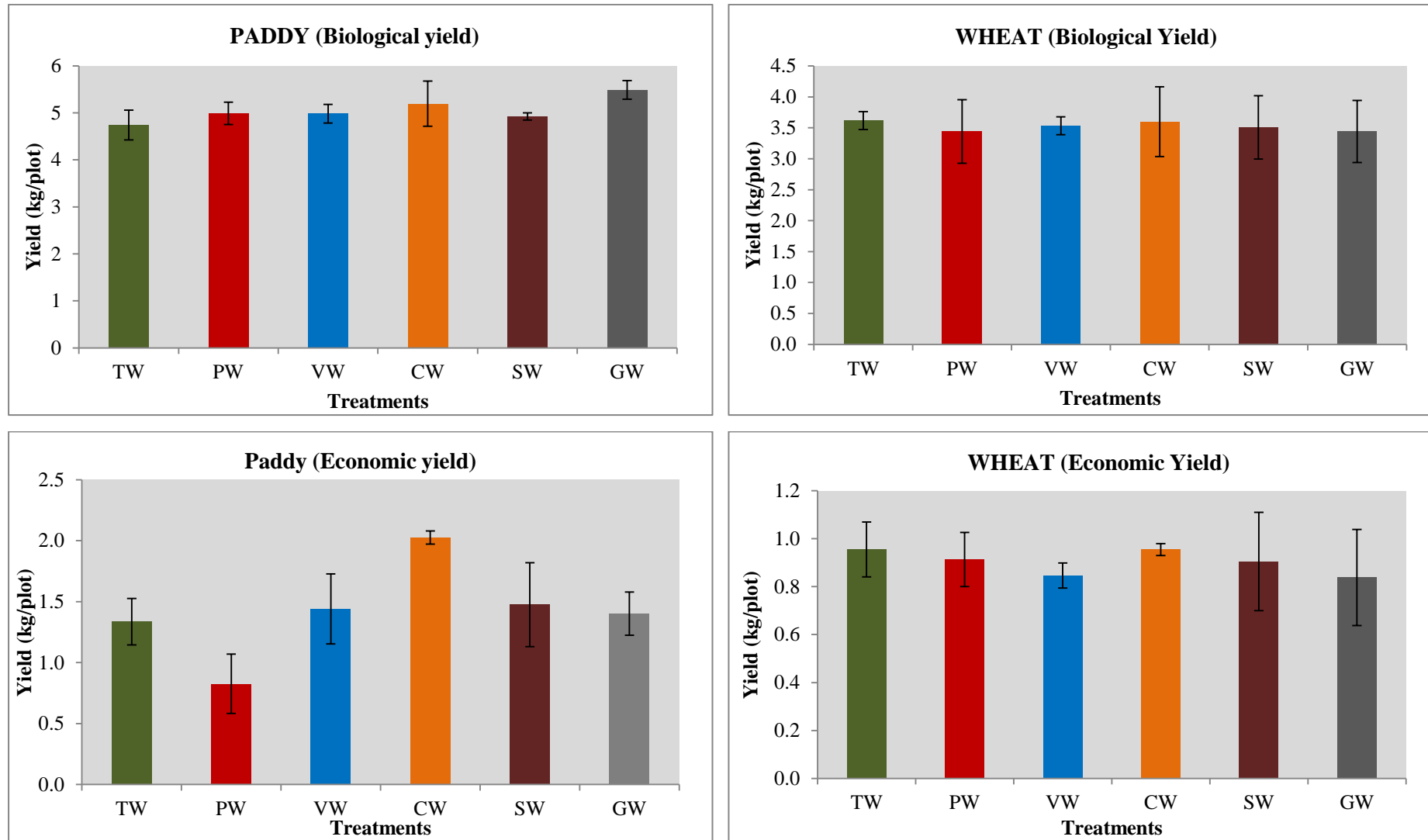


Figure 5.1: (a) Paddy (Biological yield), (b) Wheat (Biological yield), (c) Paddy (Economic yield) and (d) Wheat (Economic yield)

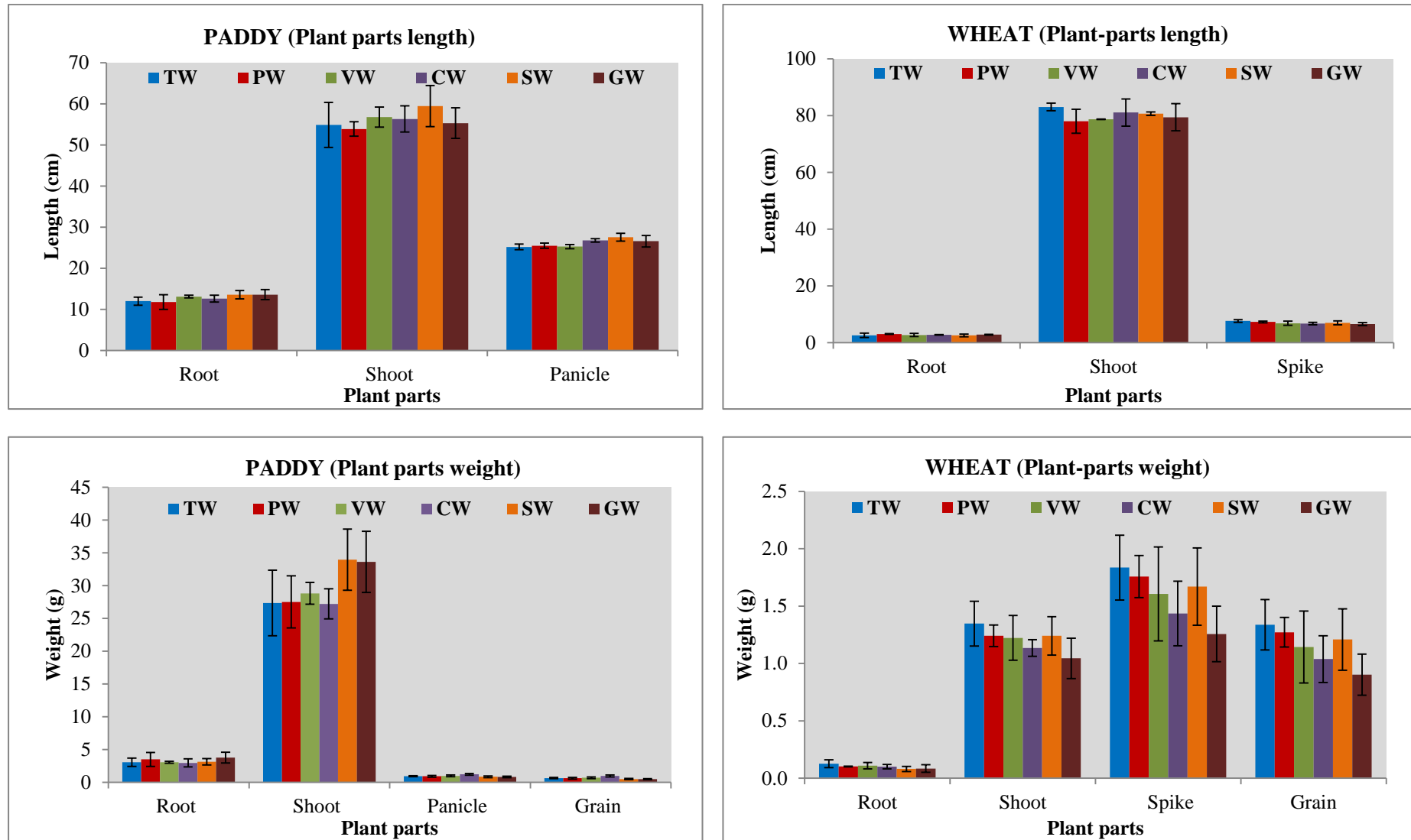


Figure 5.2: (a) Paddy (Plant parts length), (b) Wheat (Plant parts length), (c) Paddy (Plant parts dried weight) and Wheat (Plant parts dried weight)

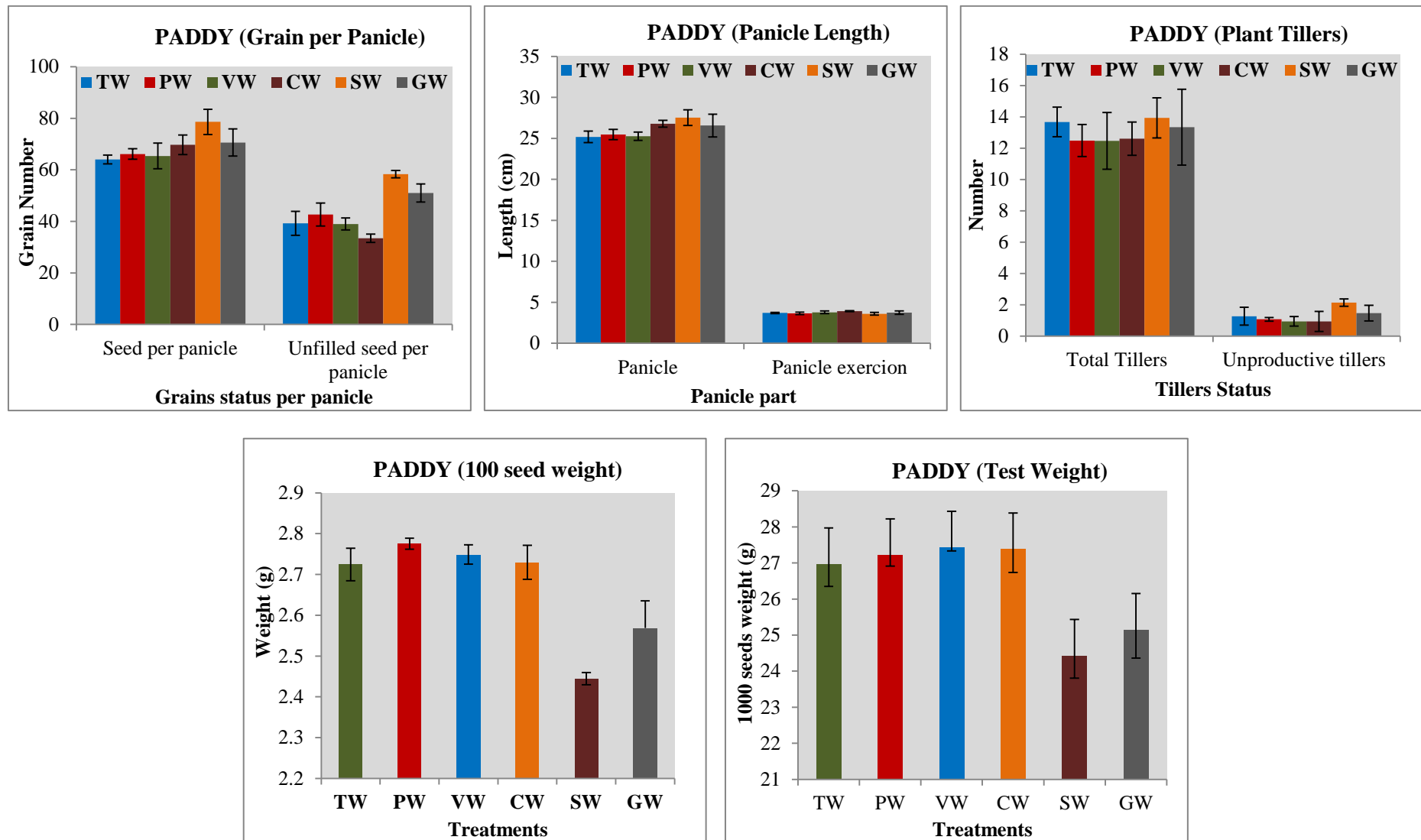


Figure 5.3: (a) Paddy (Grain per panicle), (b) Paddy (panicle length), (c) Paddy (plant tillers), (d) Paddy (100 seed weight) and (e) Paddy (Test Weight)

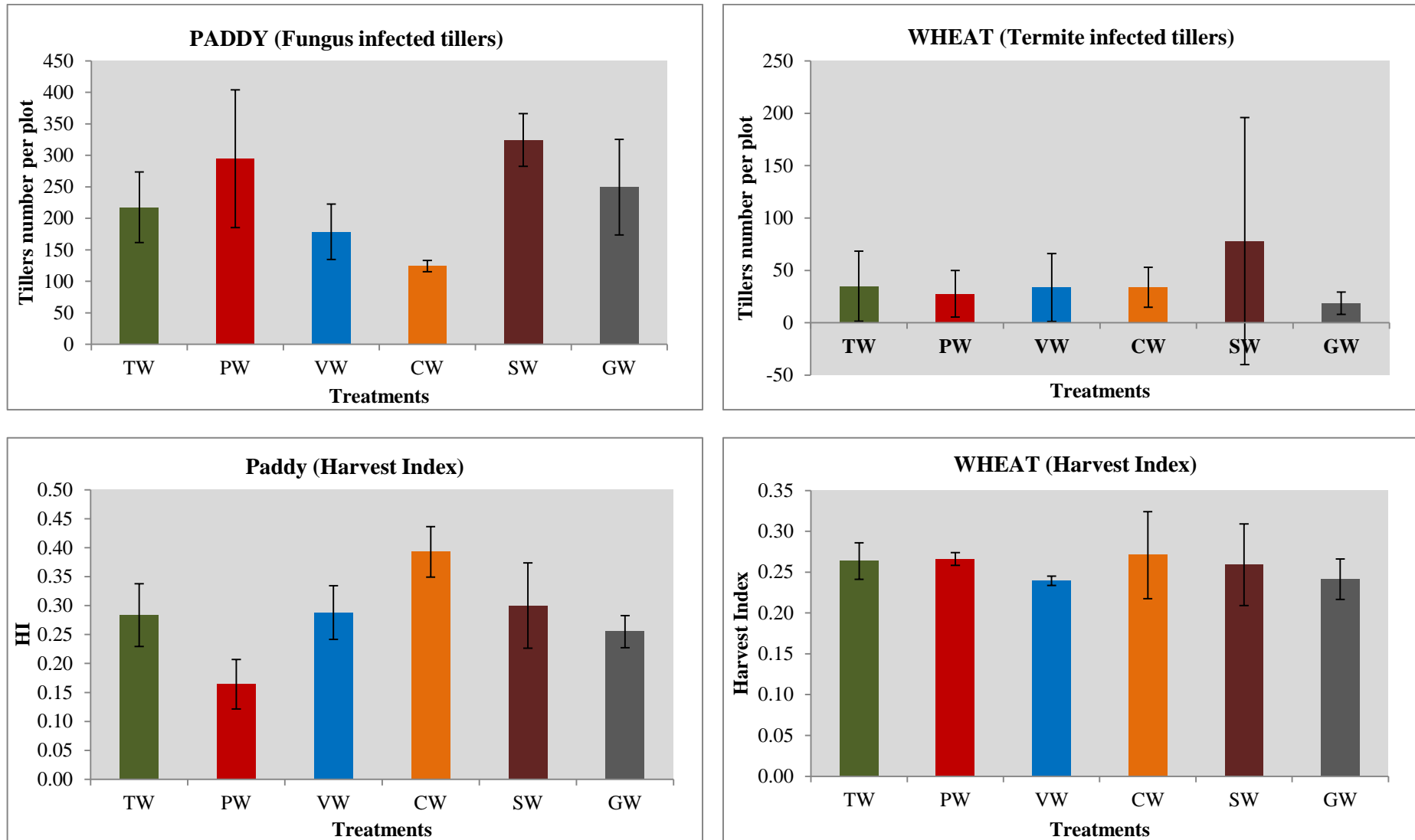


Figure 5.4: (a) Paddy (Fungus infected tillers), (b) Wheat (Termite infected tillers), (c) Paddy (Harvest Index) and (d) Wheat (Harvest Index)

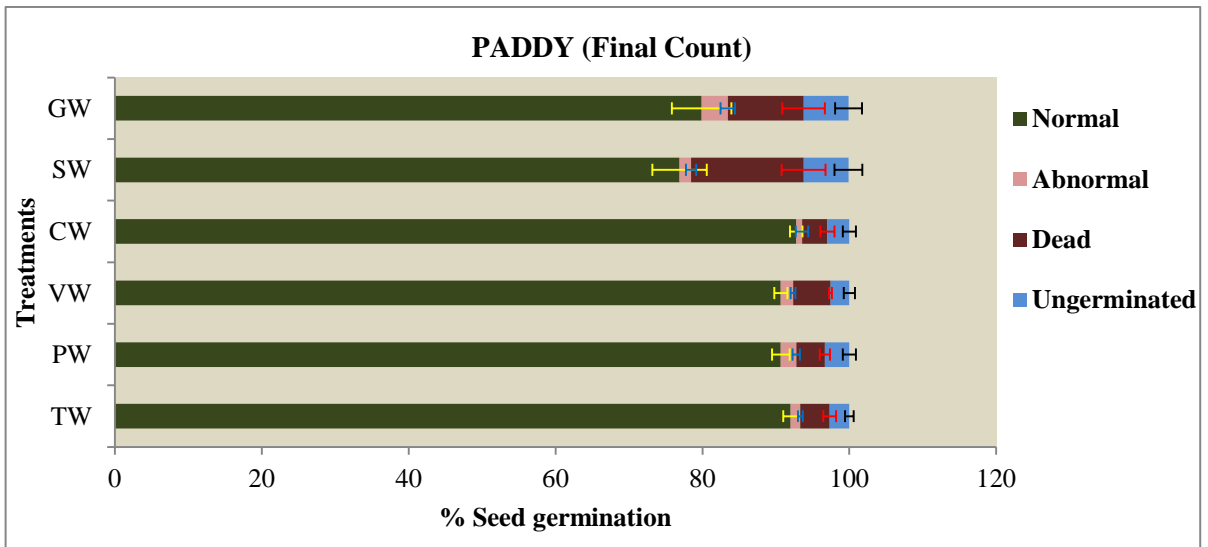
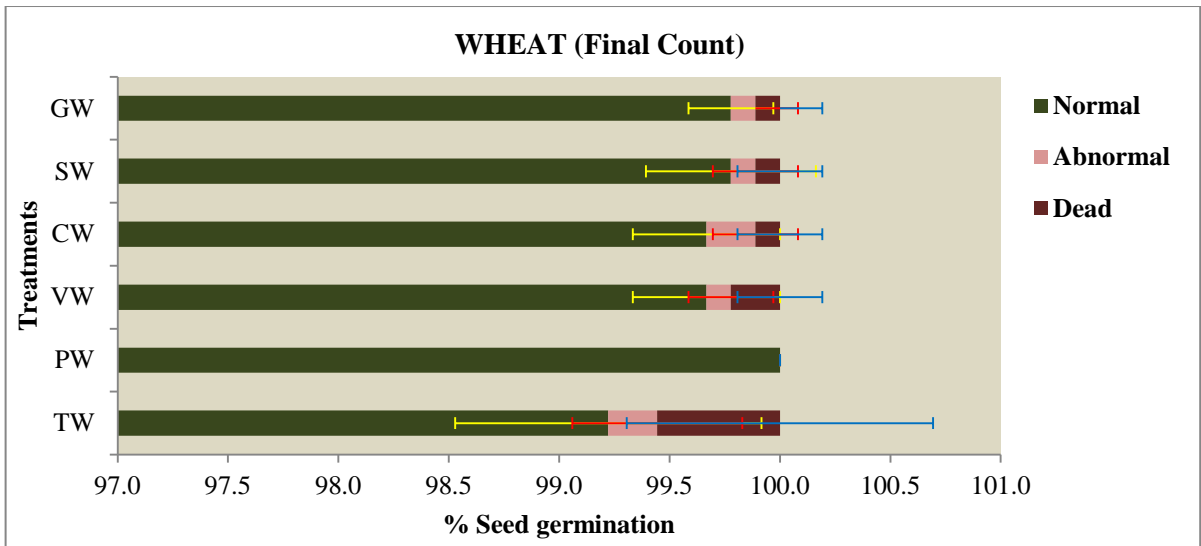
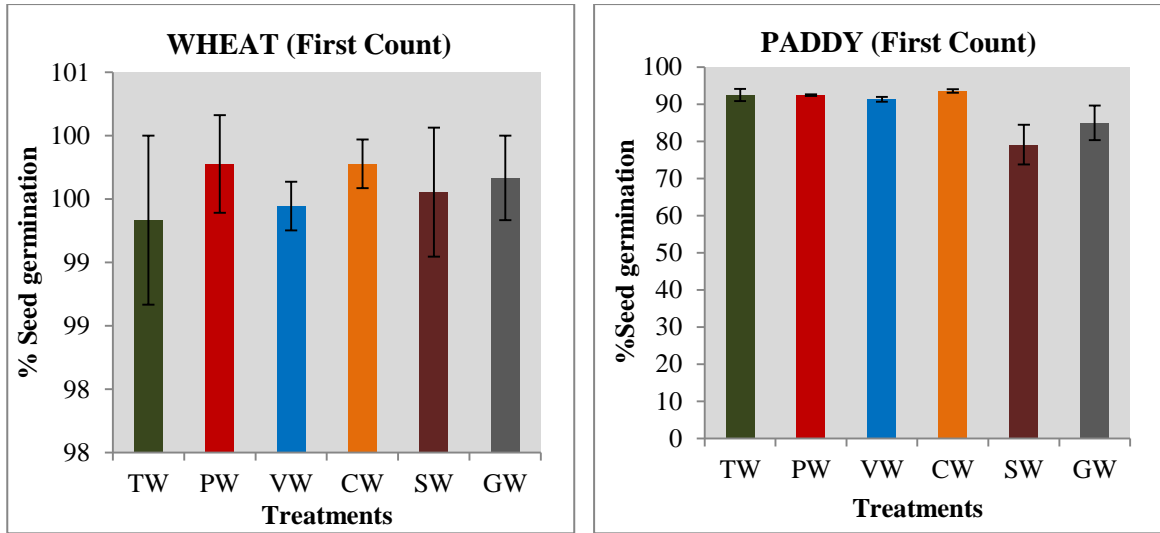


Figure 5.5: Seed germination status of Wheat and Paddy grown on different irrigation plots

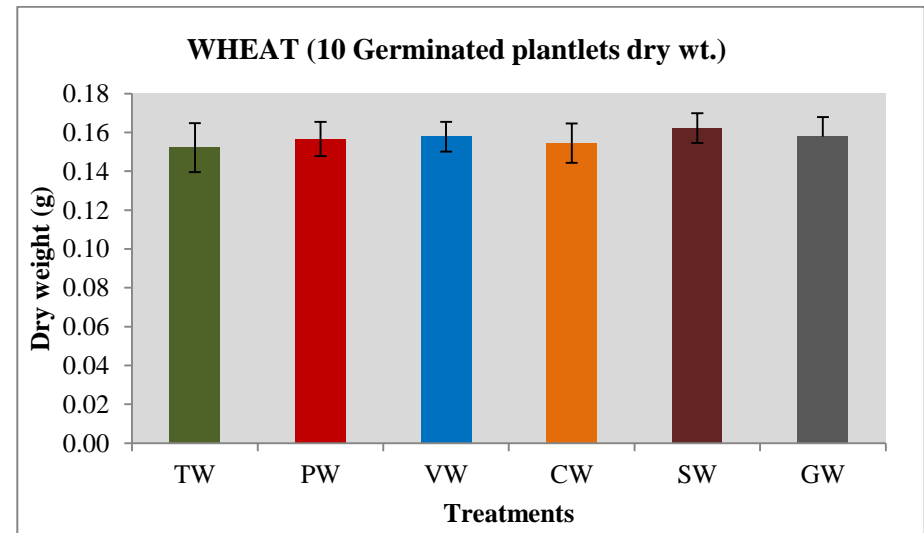
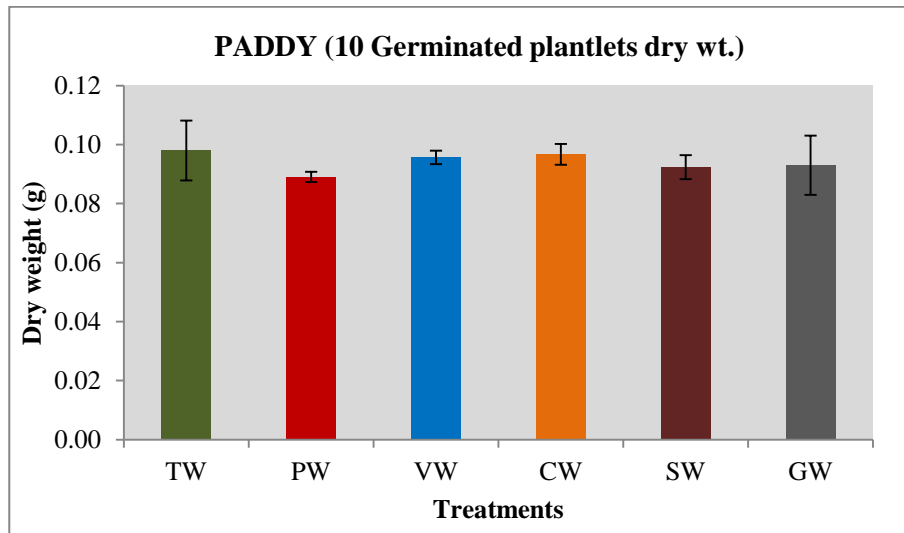
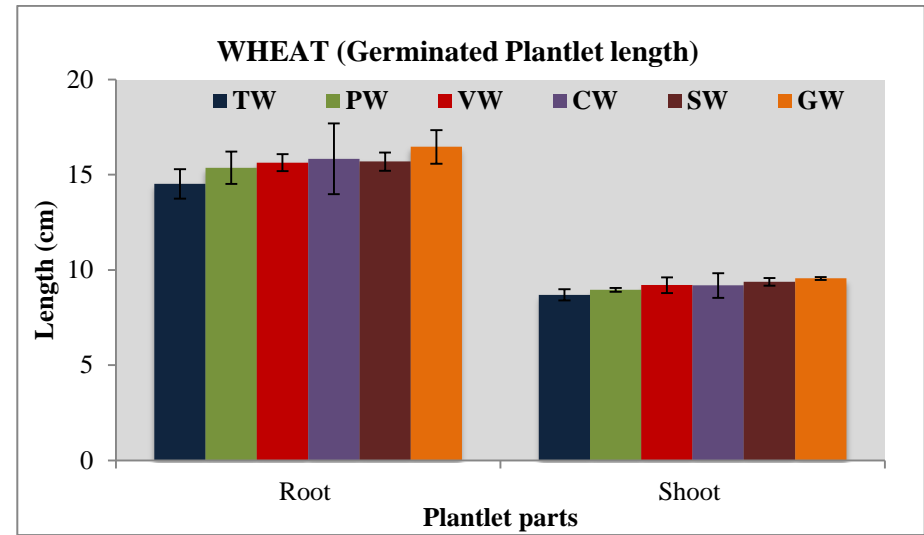
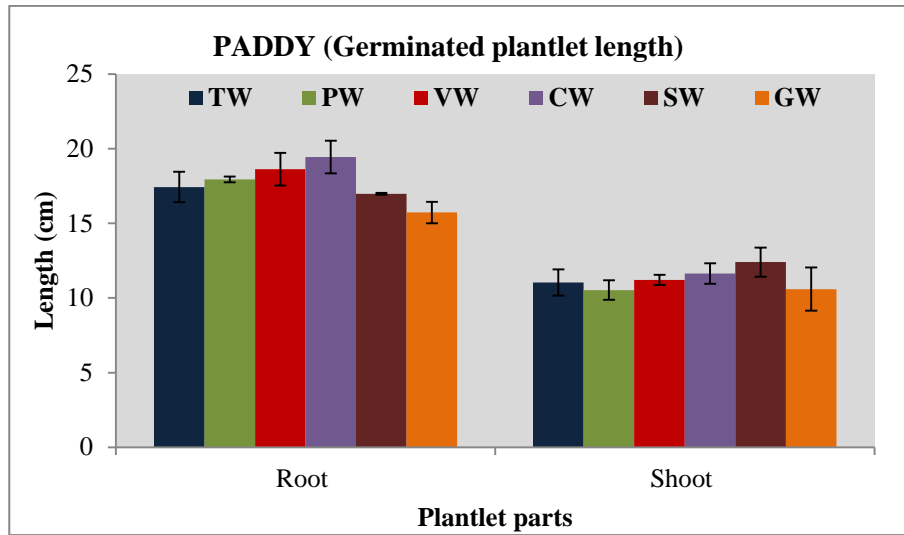


Figure 5.6: (a) Paddy (Germinated plantlets length), (b) Wheat (Germinated plantlets length), (c) Paddy (10 Germinated plantlet weight) and (d) Wheat (10 Germinated plantlet weight)

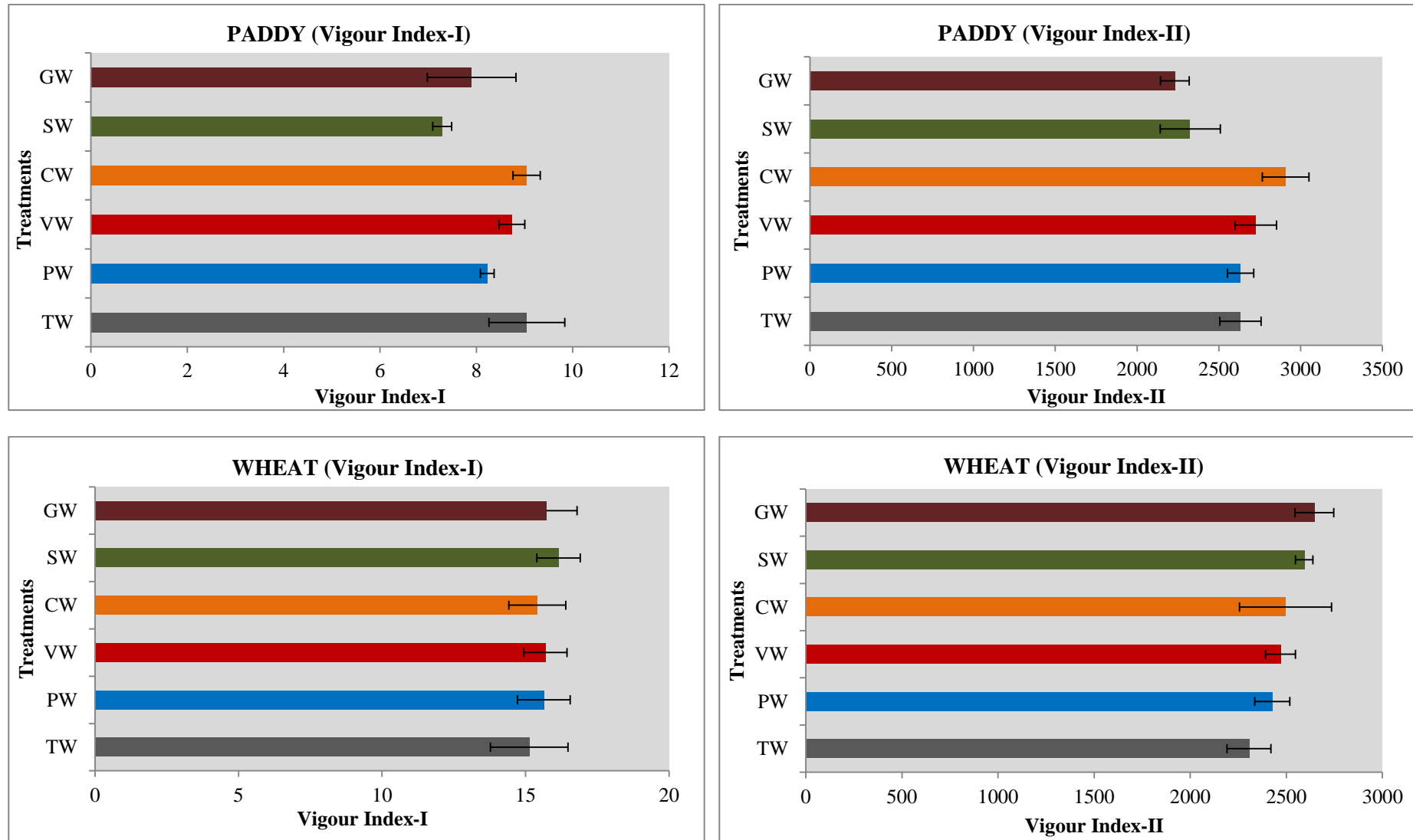


Figure 5.7: (a) Paddy (Vigour index-I), (b) Paddy (Vigour Index-II) (c) Wheat (Vigour index-I) and (d) Wheat (Vigour Index-II)

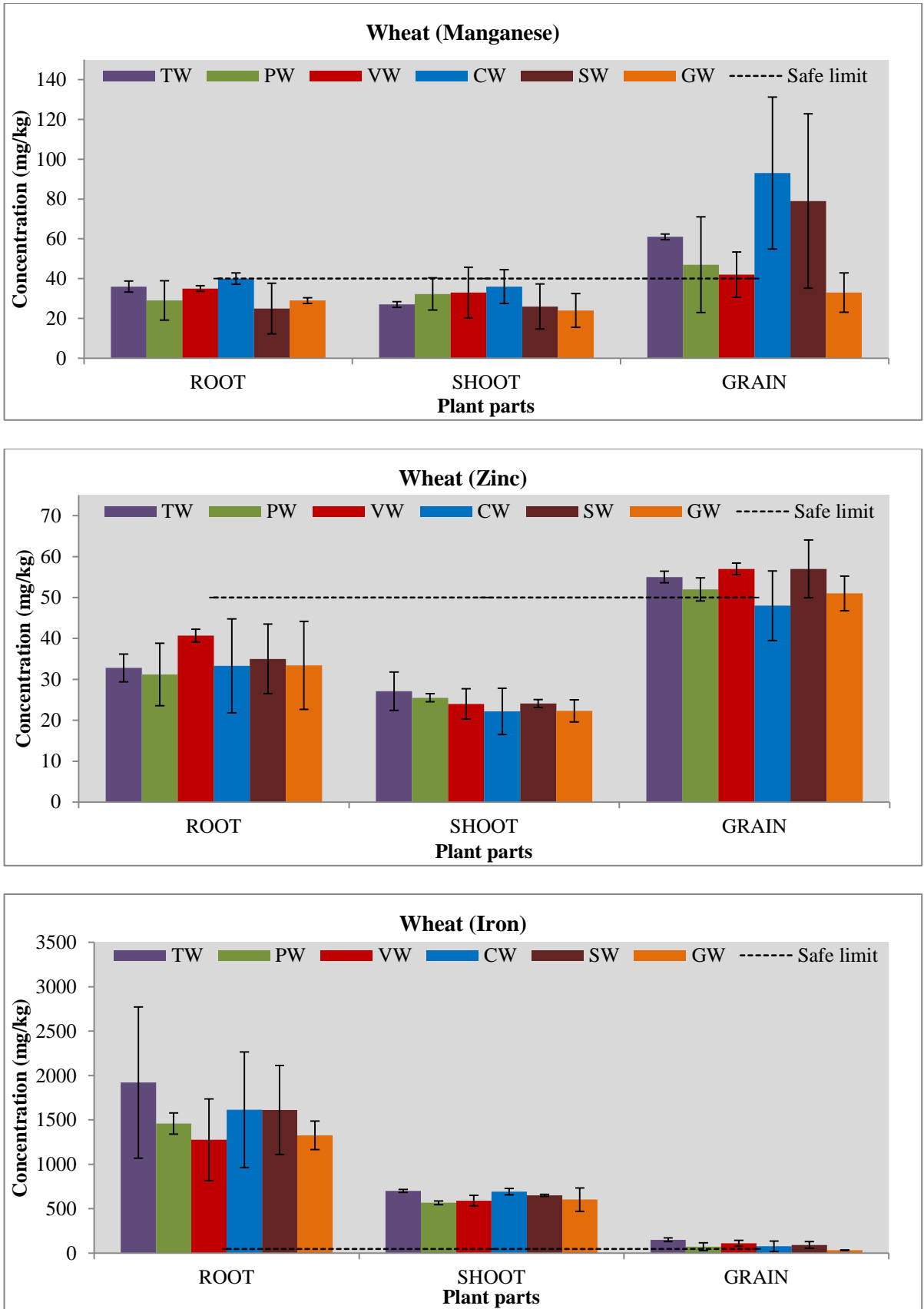


Figure 5.8: Wheat plant tissue concentration of various metals (a) Mn, (b) Zn and (c) Fe grown on different irrigation plots

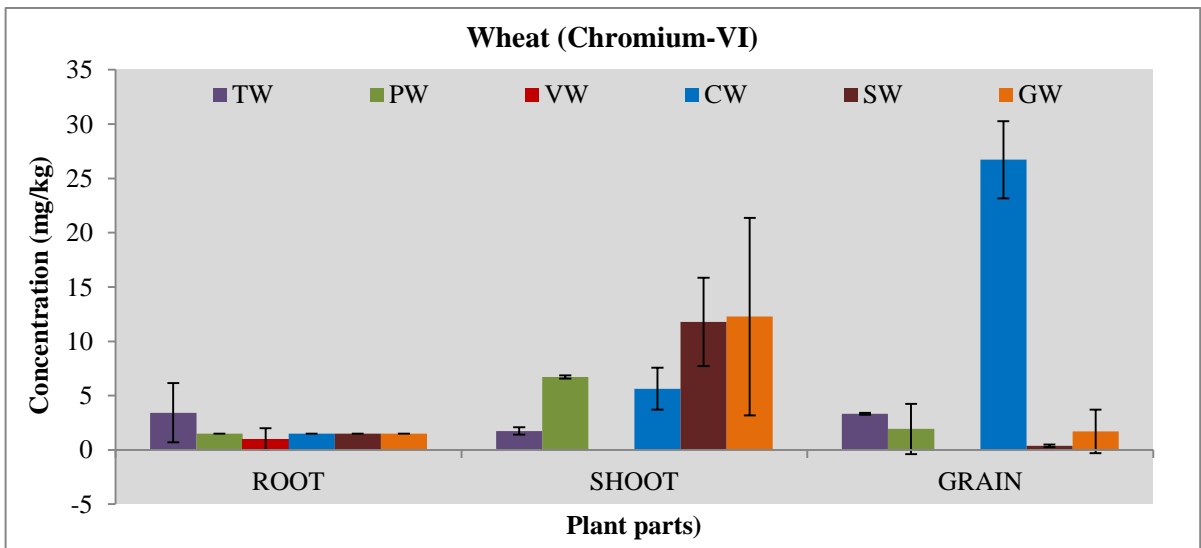
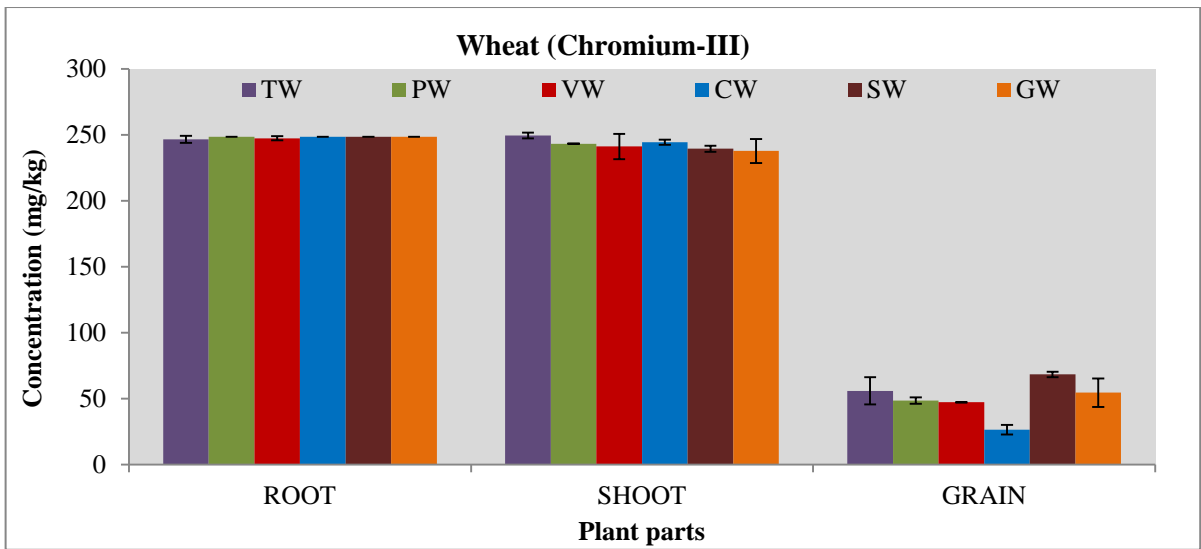
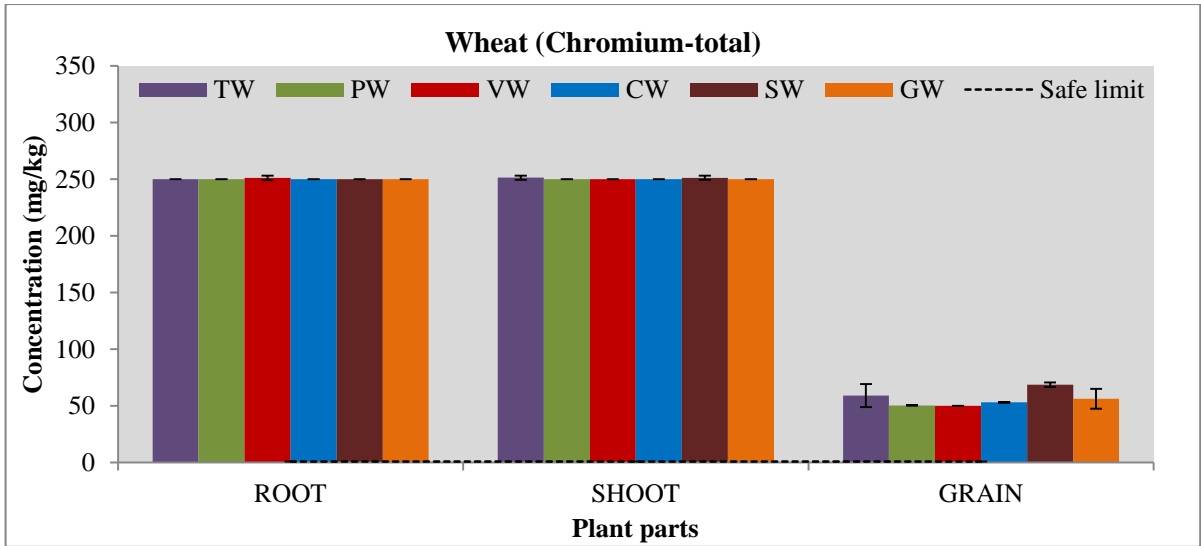


Figure 5.9: Wheat plant tissue concentration of various metals (a) Cr-total, (b) Cr-VI and (c) Cr-III grown on different irrigation plots

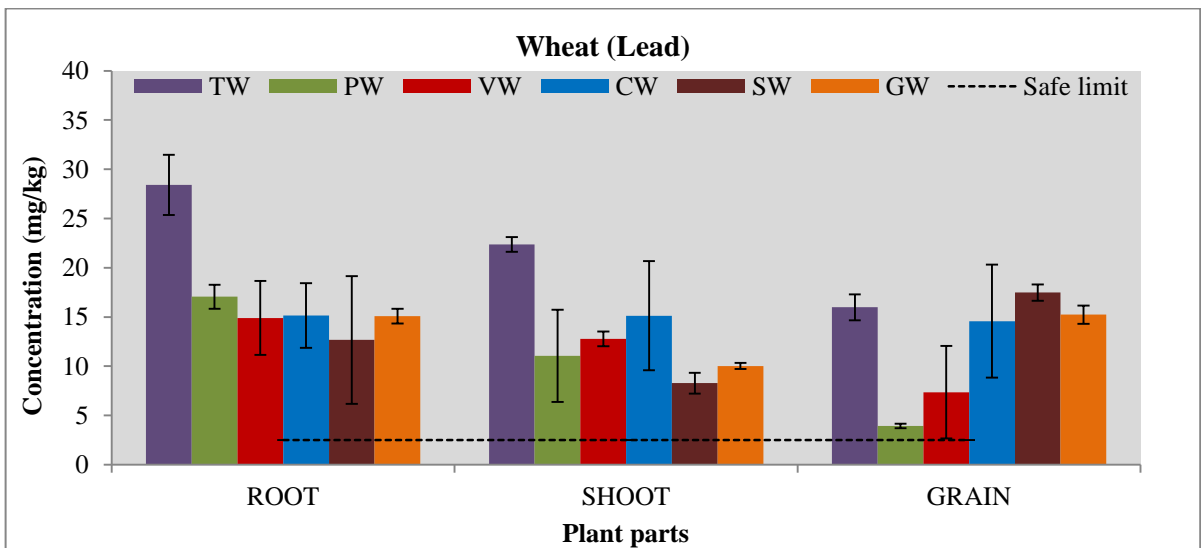
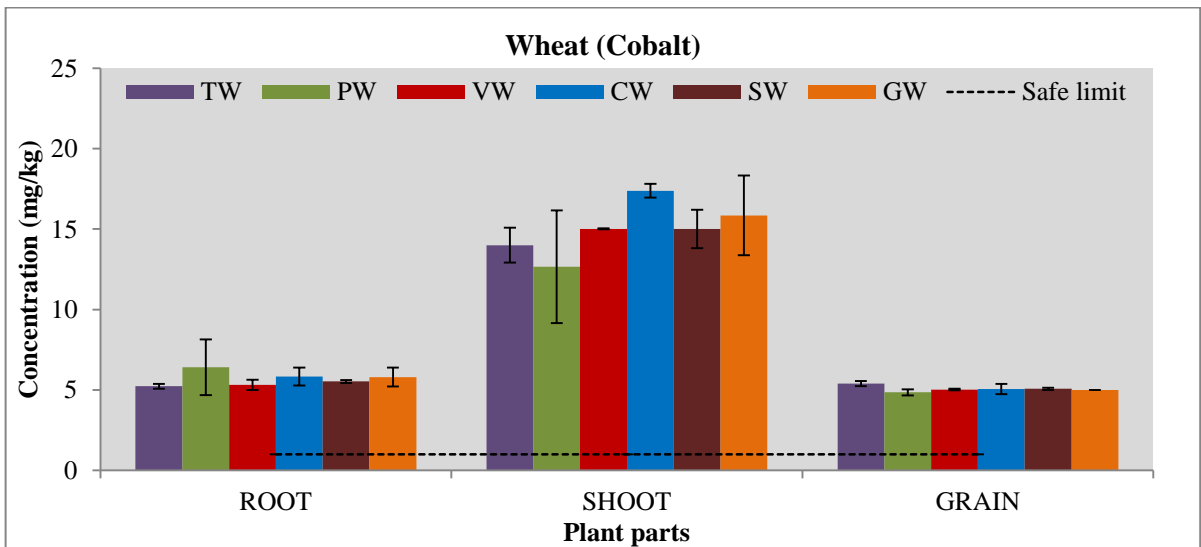
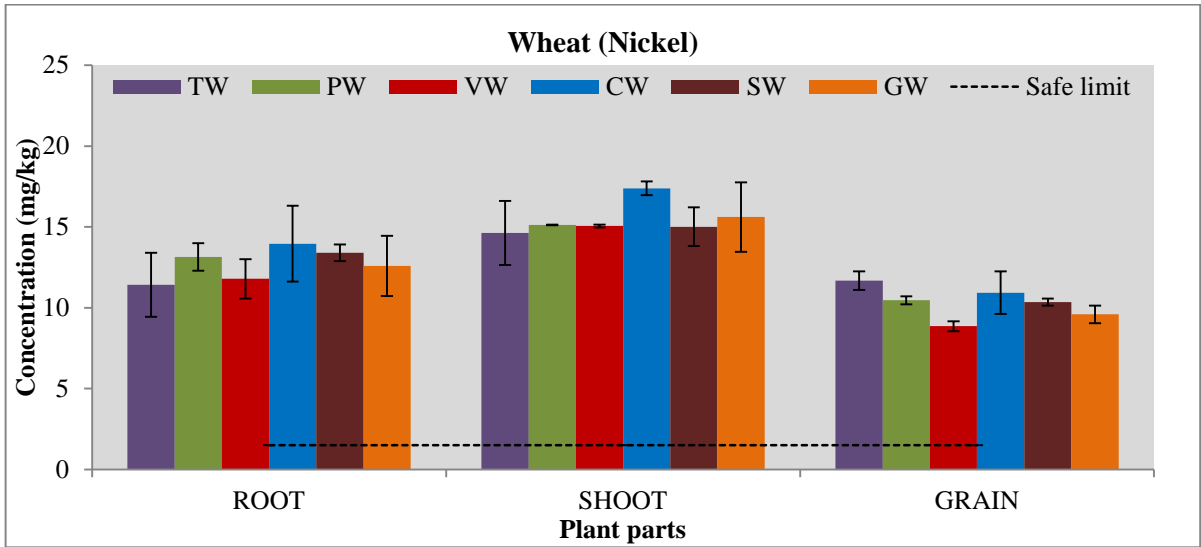


Figure 5.10: Wheat plant tissue concentration of various metal (a) Ni, (b) Pb and (c) Co grown on different irrigation plots

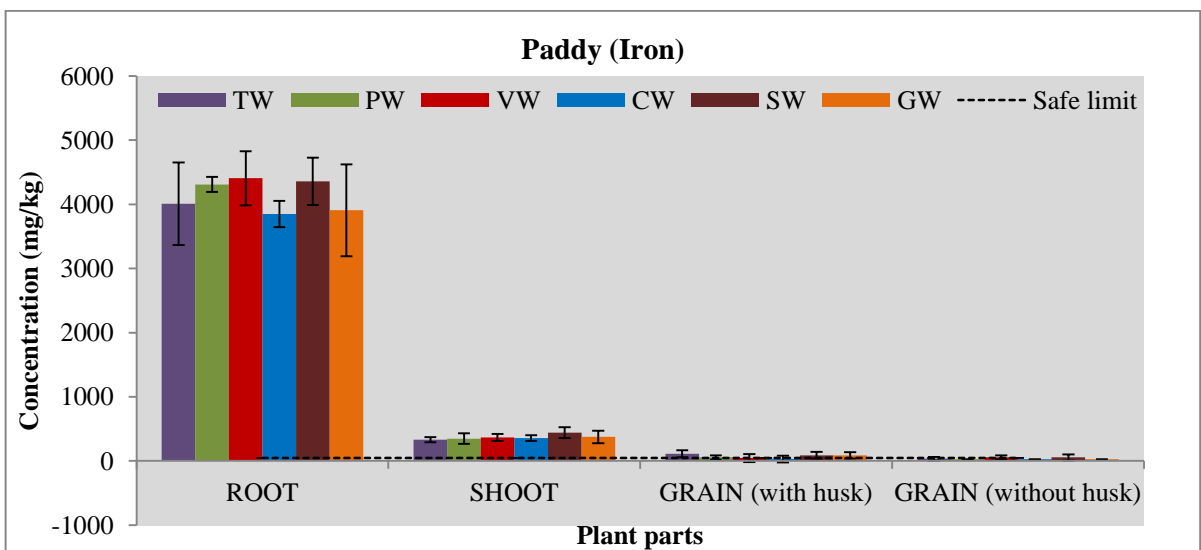
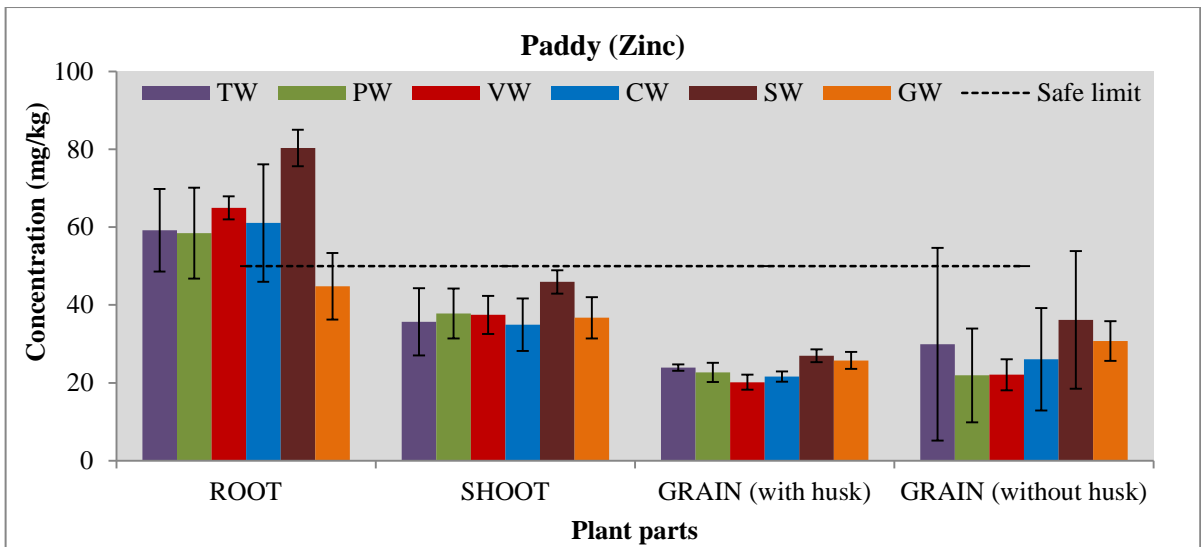
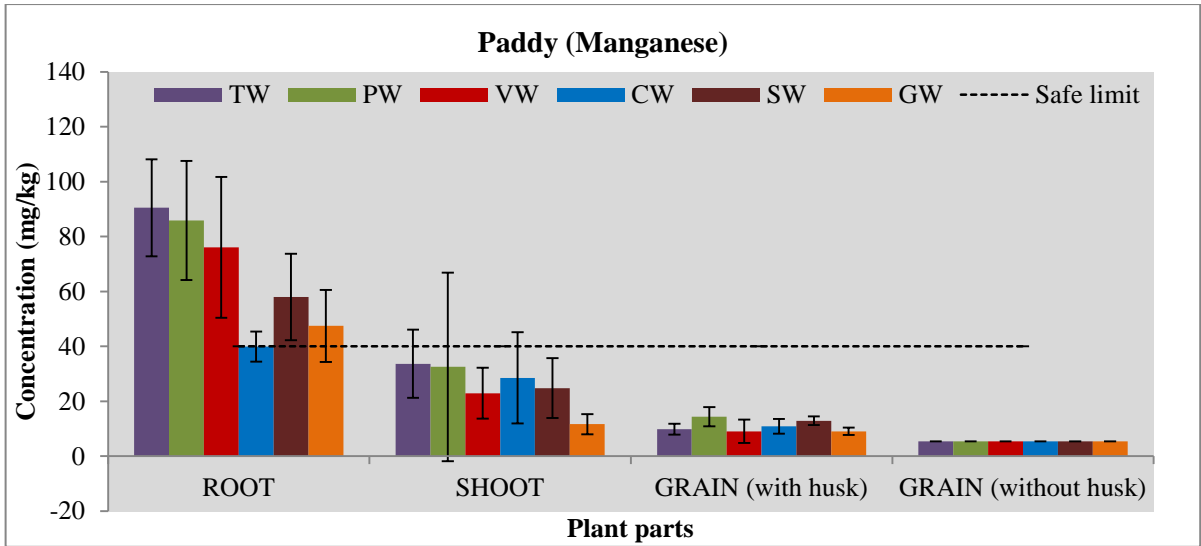


Figure 5.11: Paddy plant tissue concentration of various metal (a) Mn, (b) Zn and (c) Fe grown on different irrigation plots

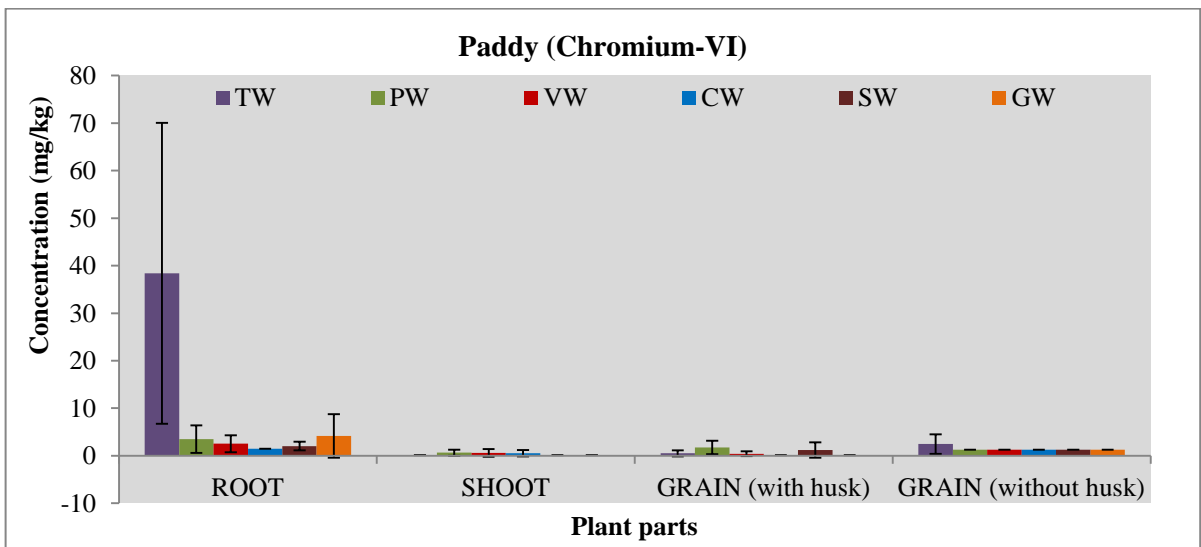
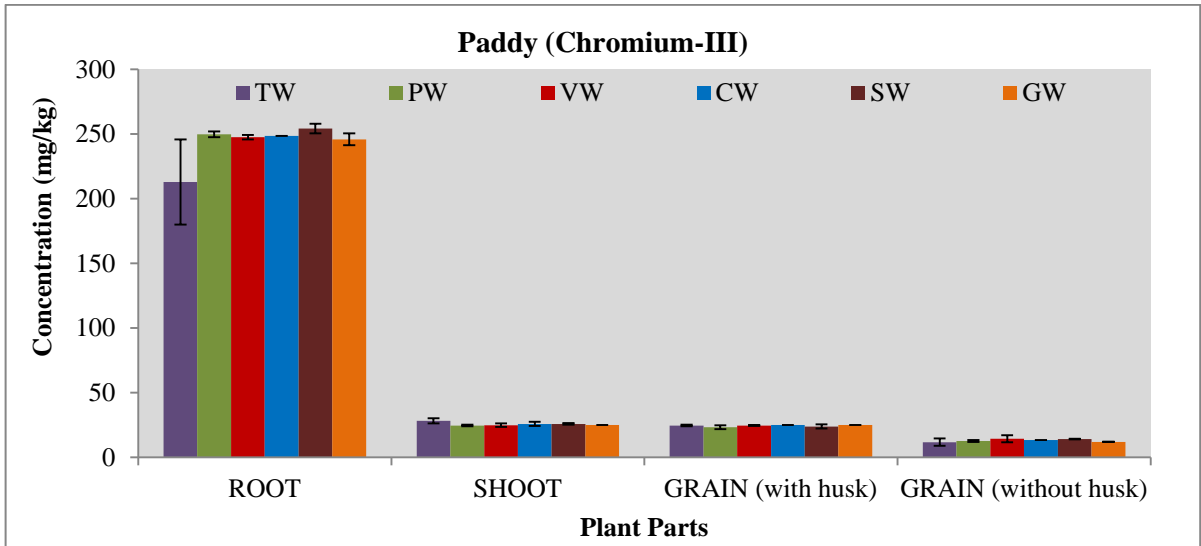
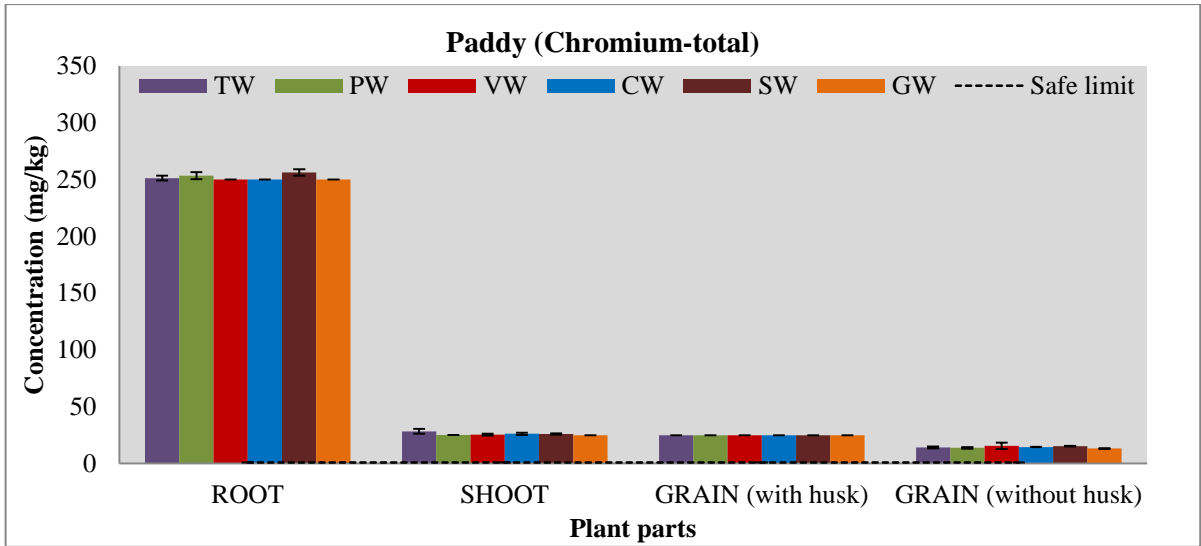


Figure 5.12: Paddy plant tissue concentration of metal (a) Cr-total, (b) Cr-VI and (c) Cr-III grown on different irrigation plots

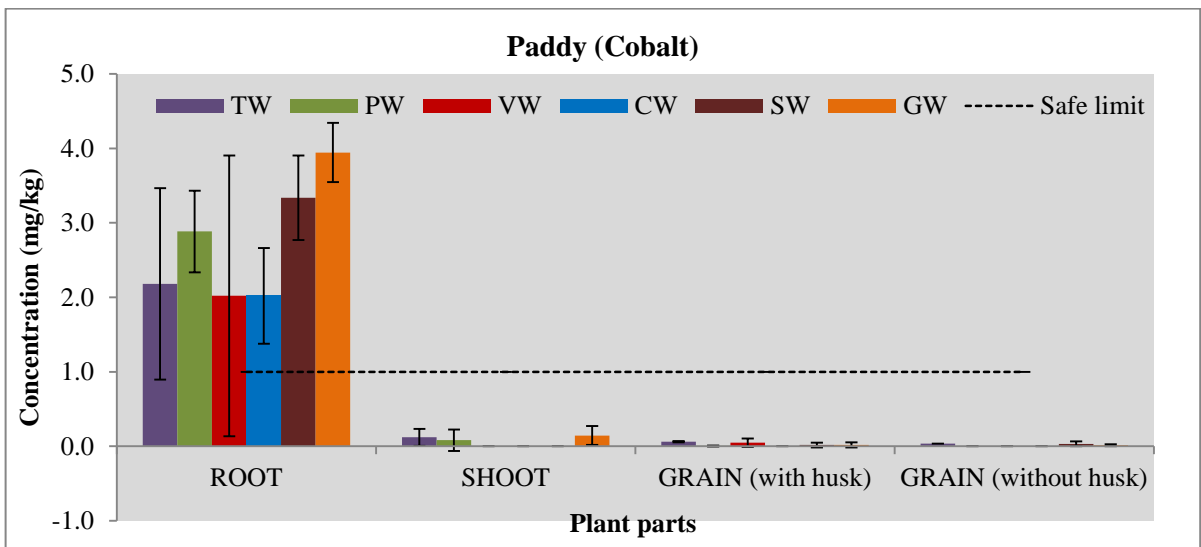
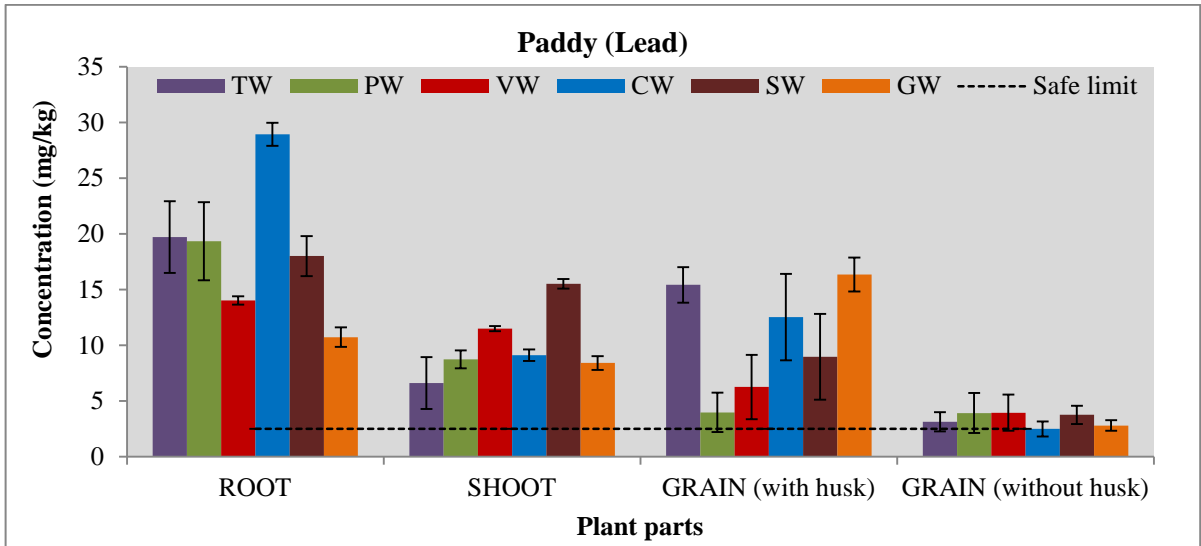
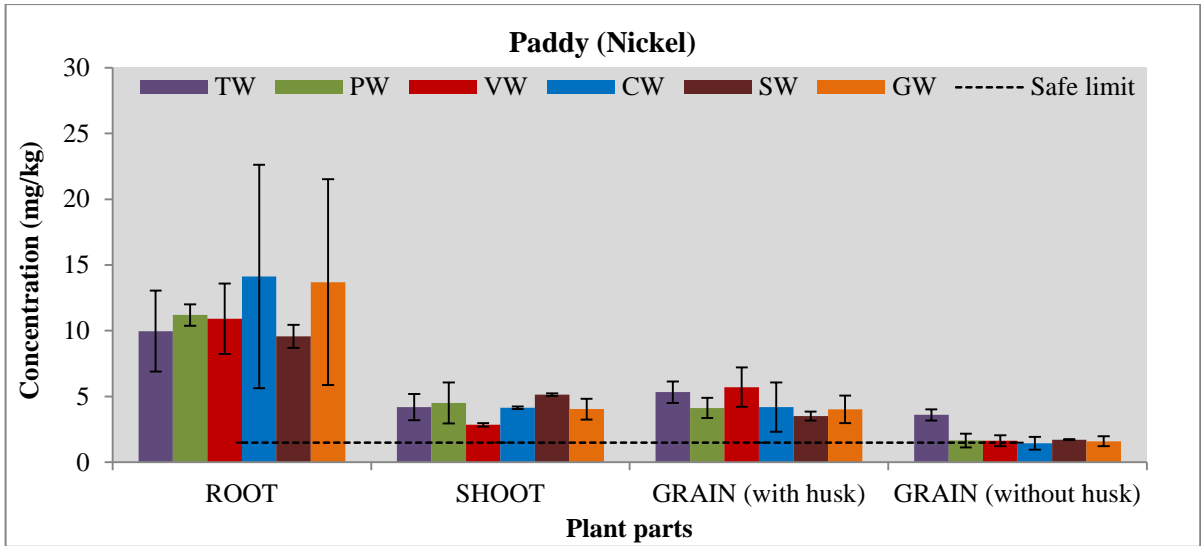


Figure 5.13: Paddy plant tissue concentration of various metal (a) Ni, (b) Pb and (c) Co grown on different irrigation plots

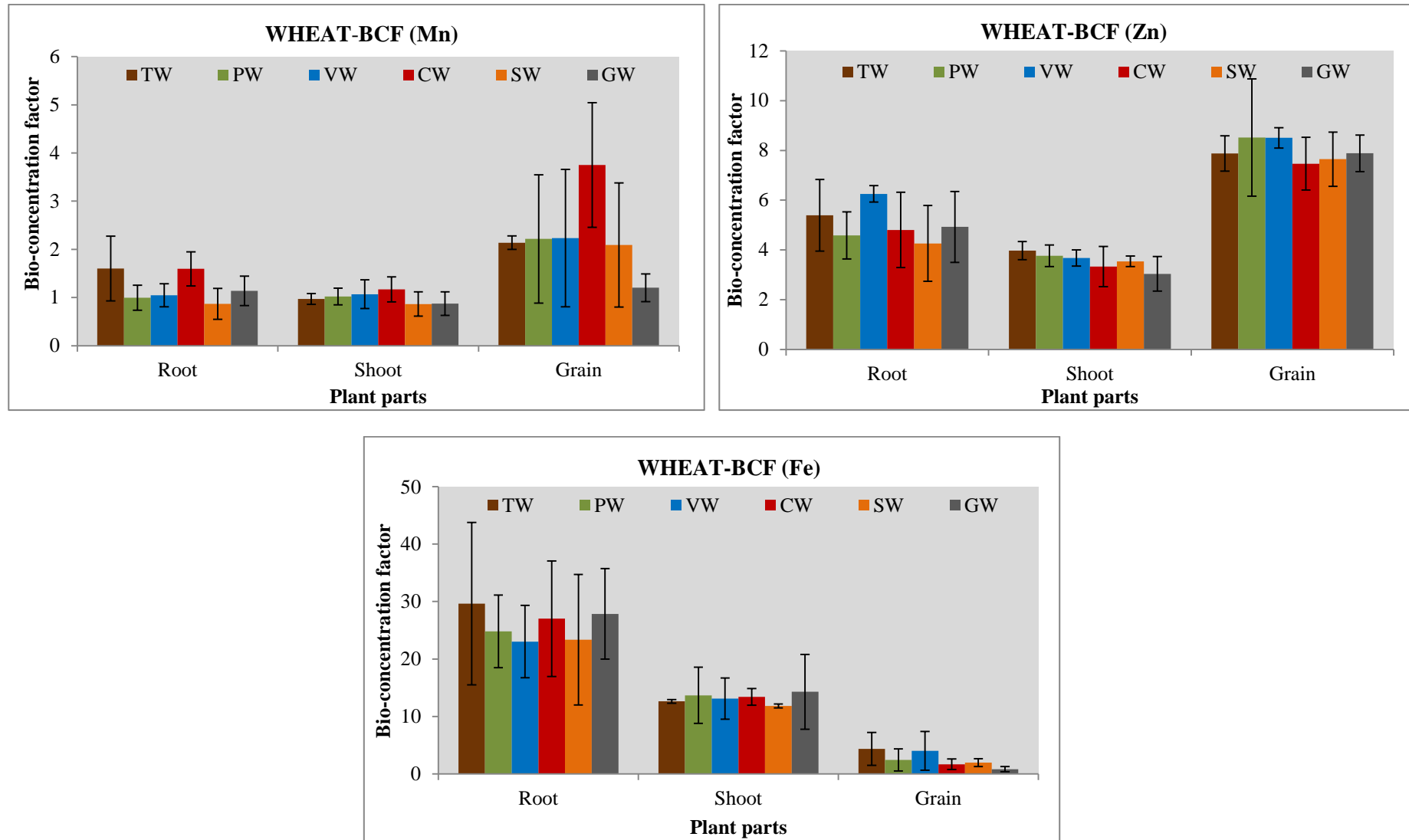


Figure 5.14: Bio-concentration factor of various metals (a) Manganese, (b) Zinc and (c) Iron in Wheat plant grown on different irrigation plots

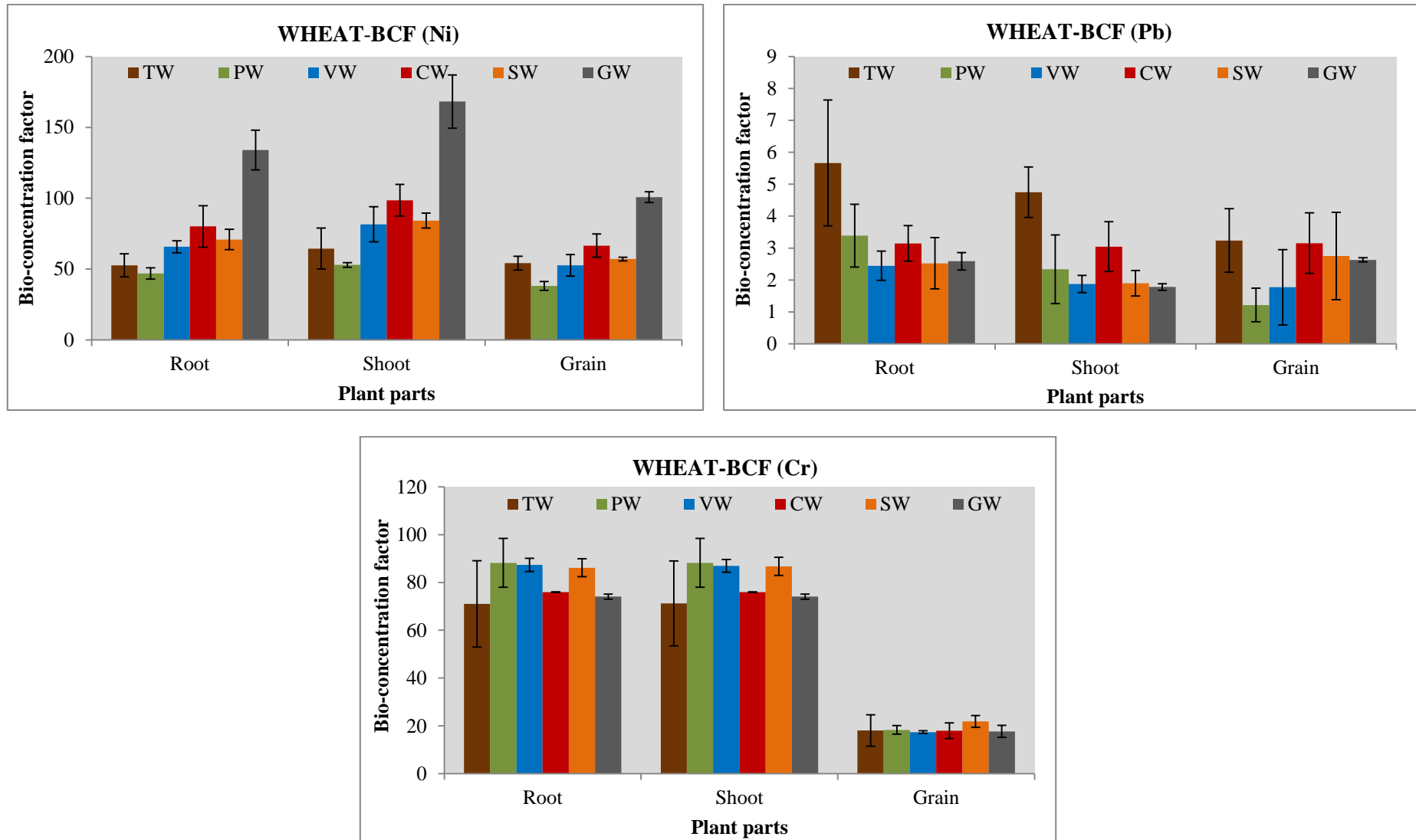


Figure 5.15: Bio-concentration factor of various metals (a) Nickel, (b) Lead and (c) Chromium in Wheat plant grown on different irrigation plots

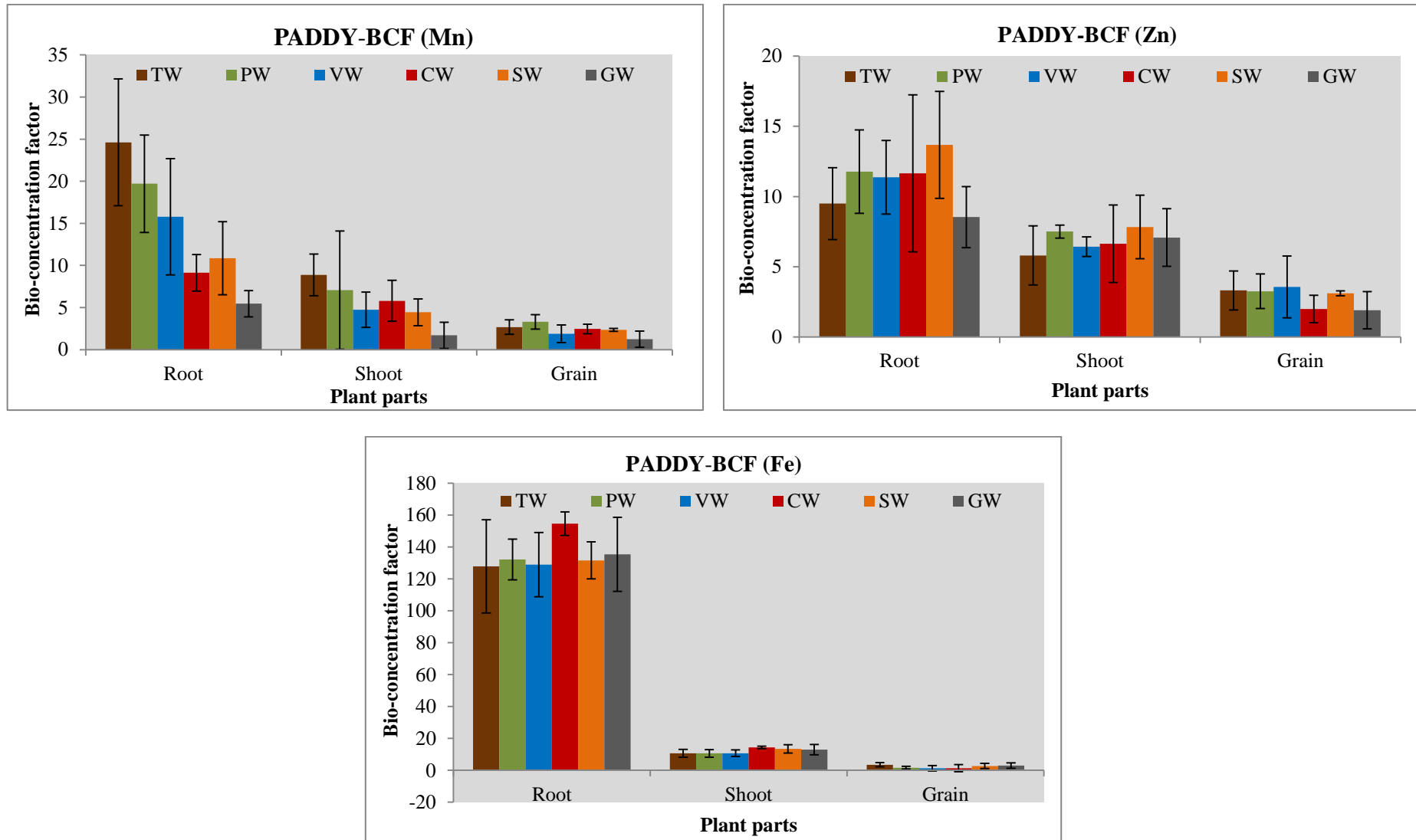


Figure 5.16: Bio-concentration factor of various metals (a) Manganese, (b) Zinc and (c) Iron in Paddy plant grown on different irrigation plots

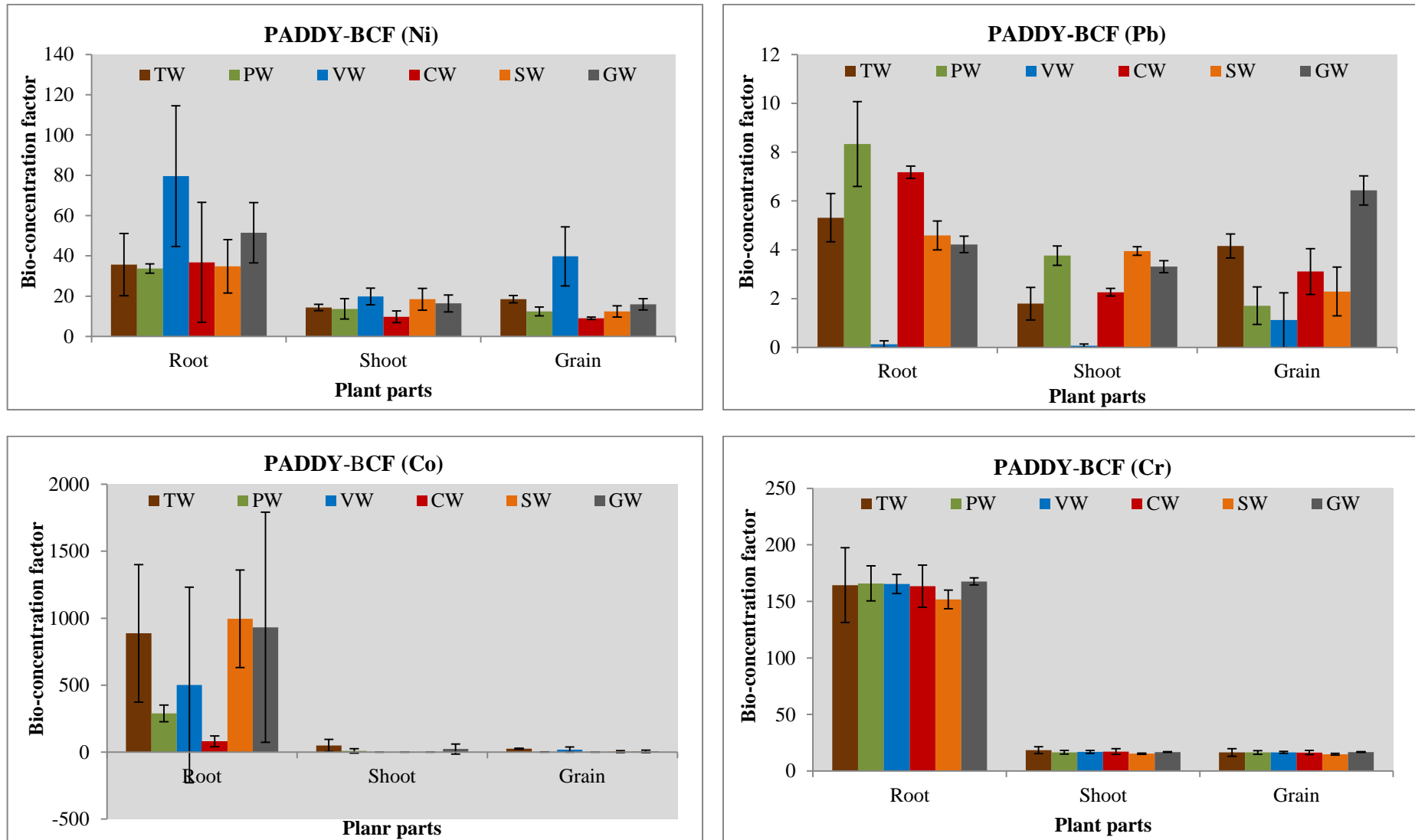


Figure 5.17: Bio-concentration factor of various metals (a) Nickel, (b) Lead, (c) Cobalt and (d) Chromium in Paddy grown on different irrigation plots

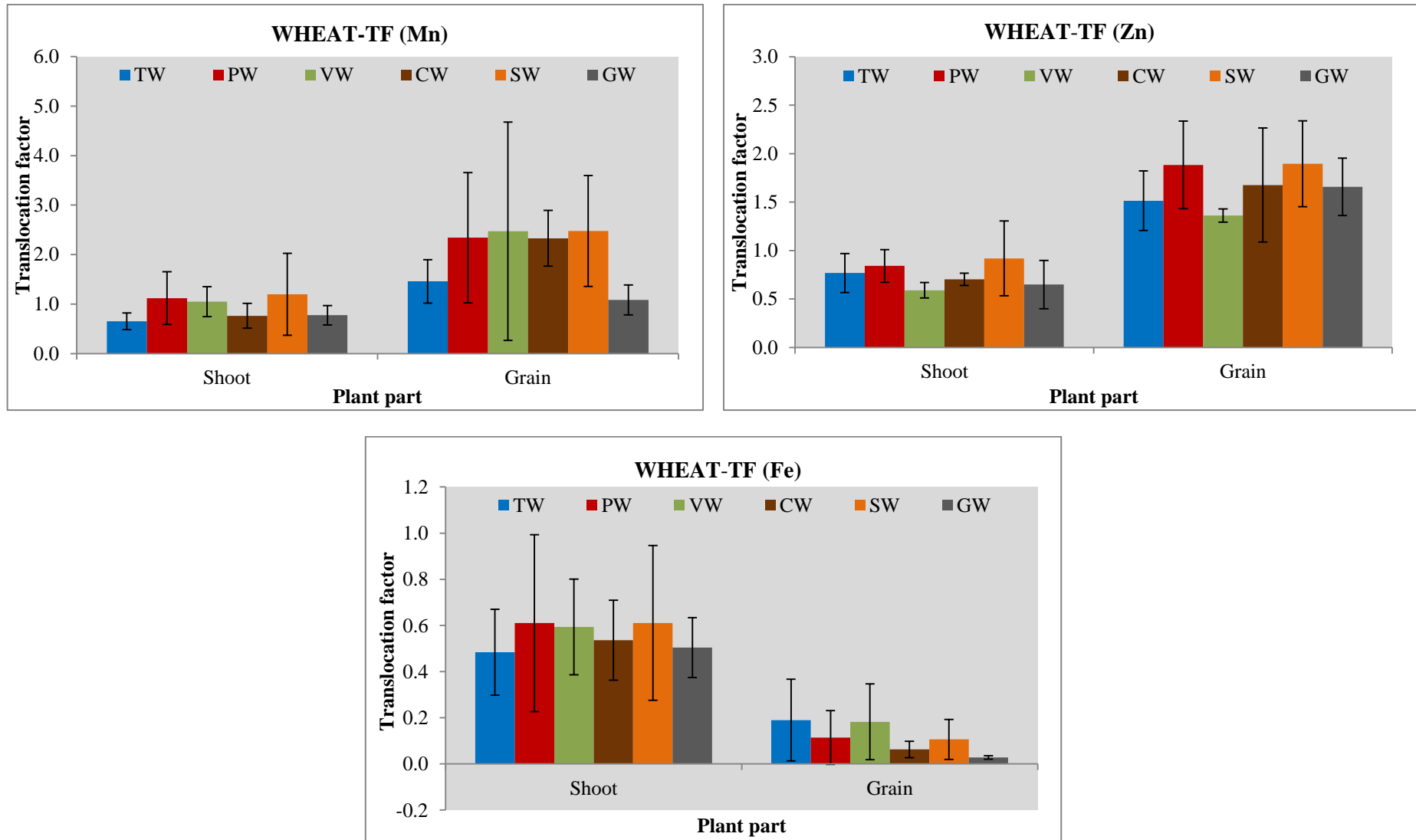


Figure 5.18: Translocation factor of various metals (a) Manganese, (b) Zinc and (c) Iron in Wheat grown on different irrigation plots

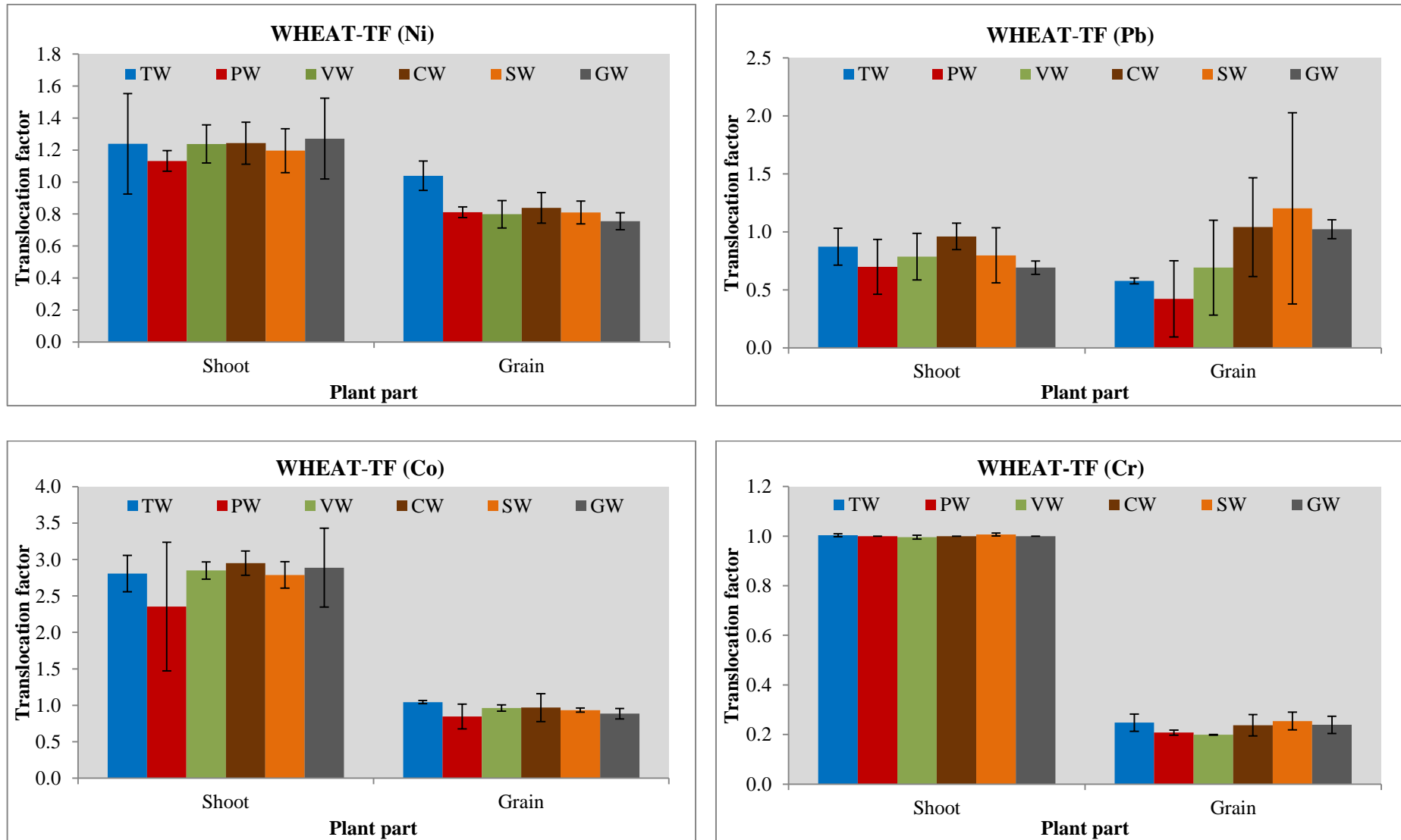


Figure 5.19: Translocation factor of various metals (a) Nickel, (b) Lead, (c) Cobalt and (d) Chromium in Wheat grown on different irrigation plots

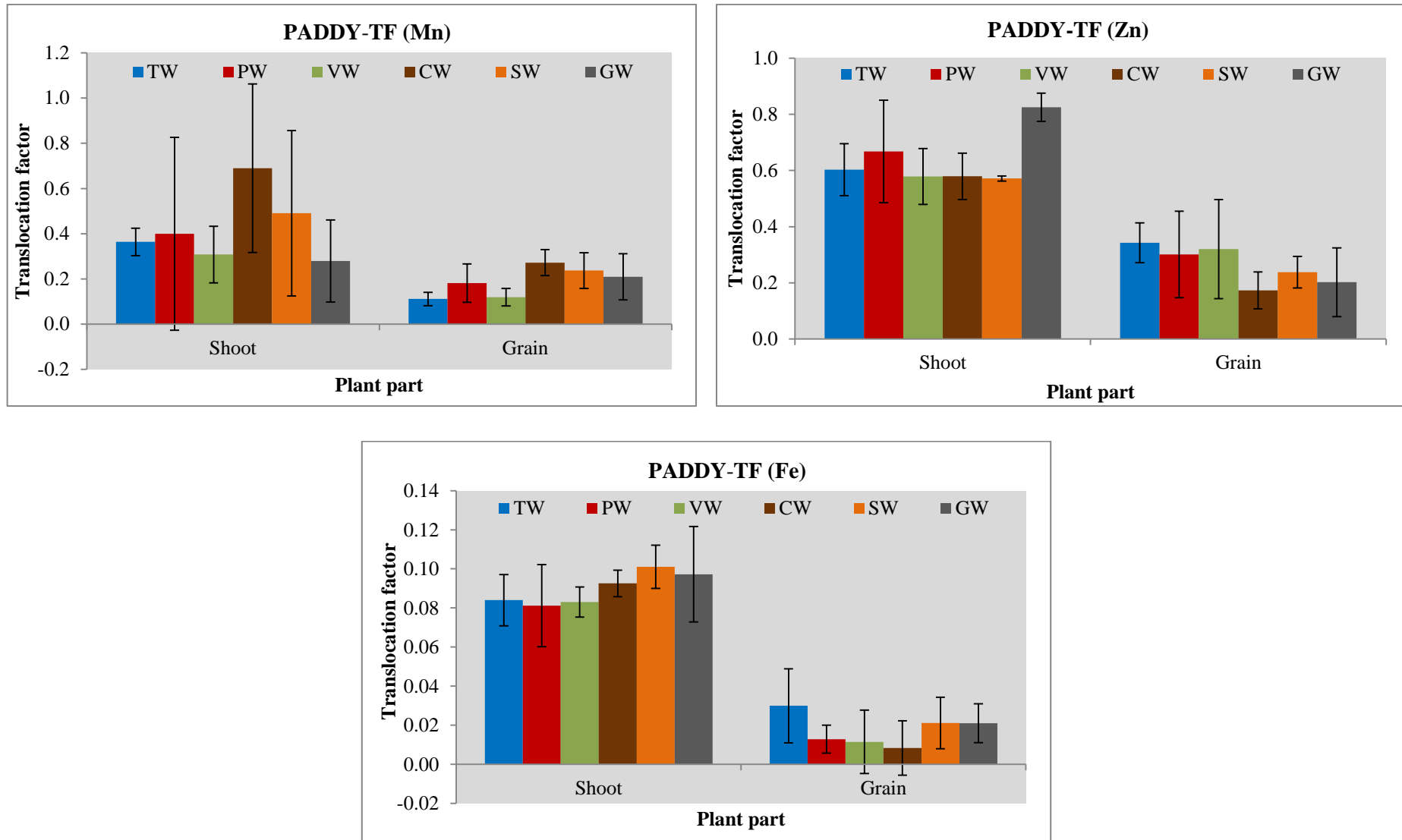


Figure 5.20: Translocation factor of various metals (a) Manganese, (b) Zinc and (c) Iron in Paddy grown on different irrigation plots

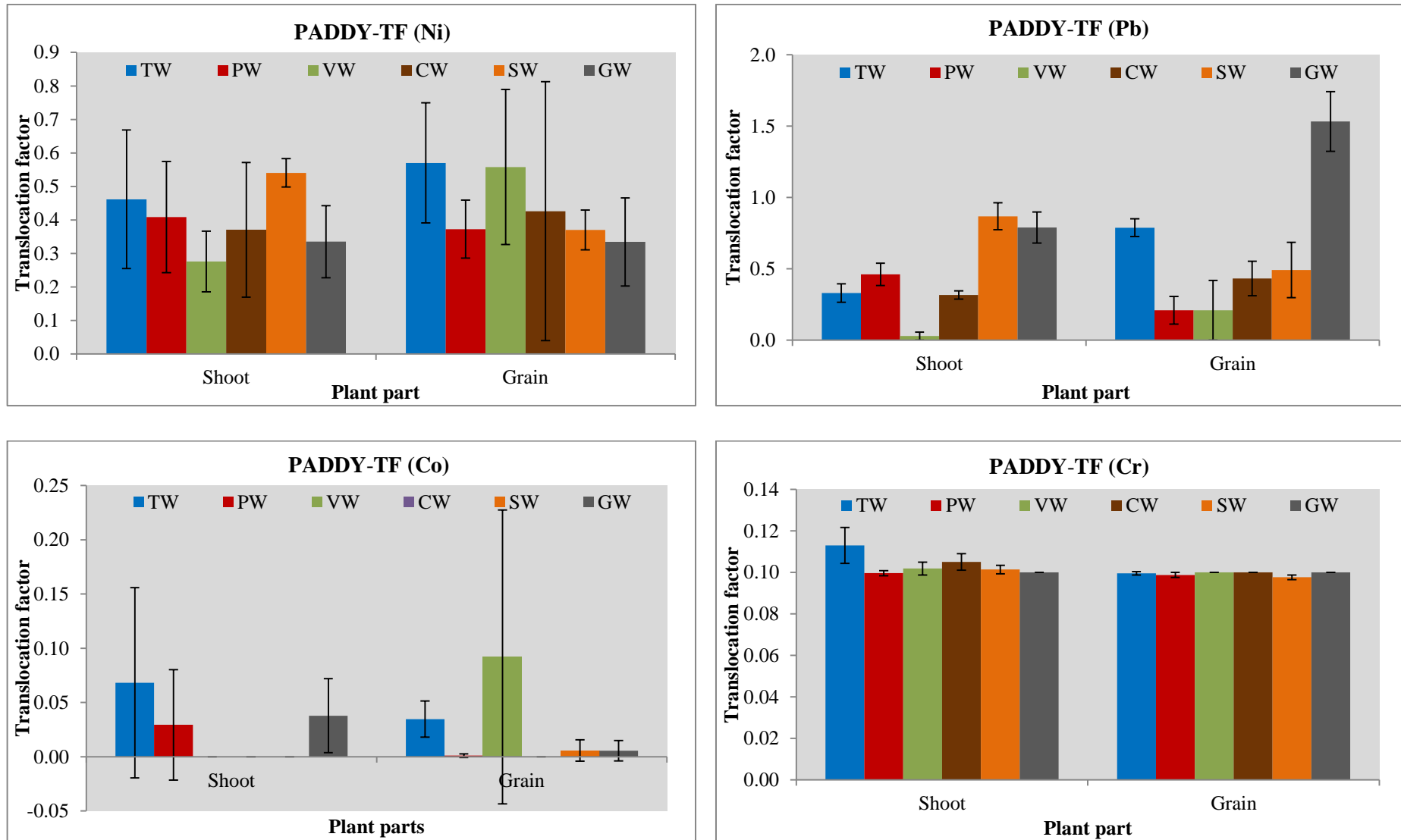


Figure 5.21: Translocation factor of various metals (a) Nickel, (b) Lead, (c) Cobalt and (d) Chromium in Paddy grown on different irrigation plots

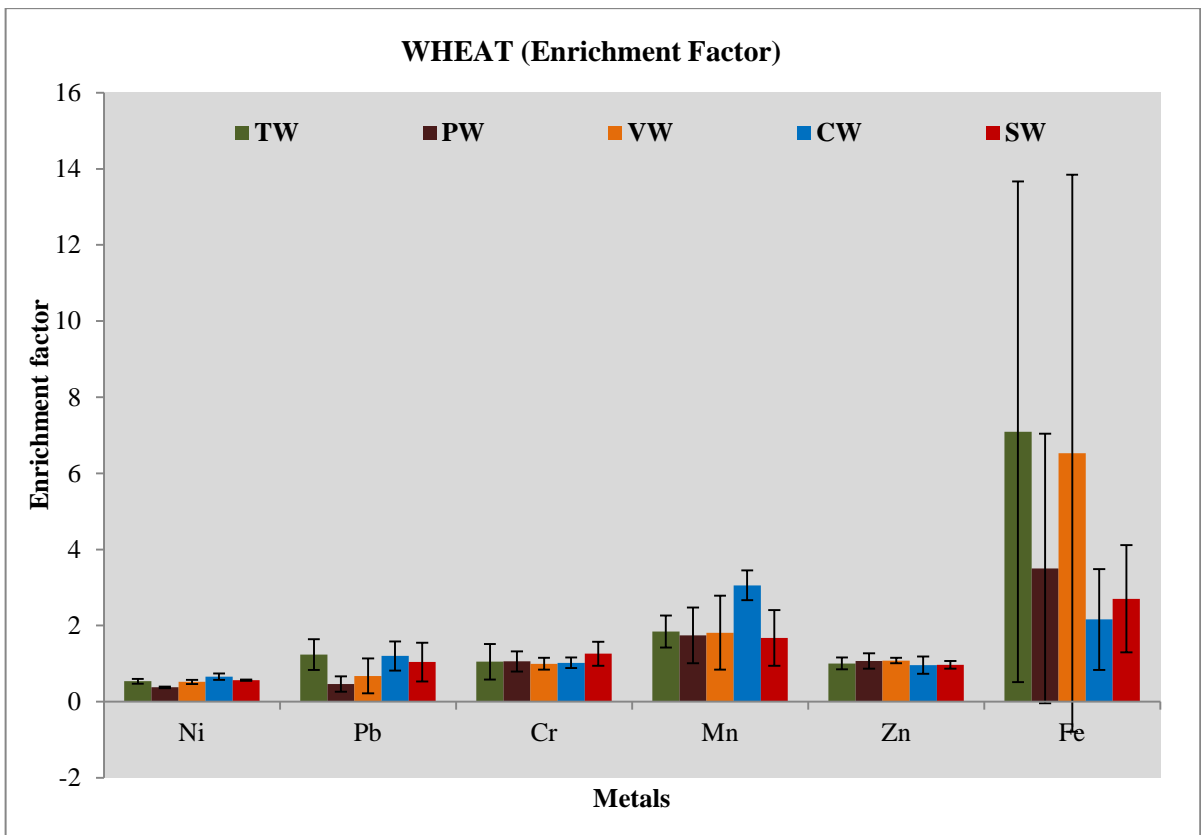
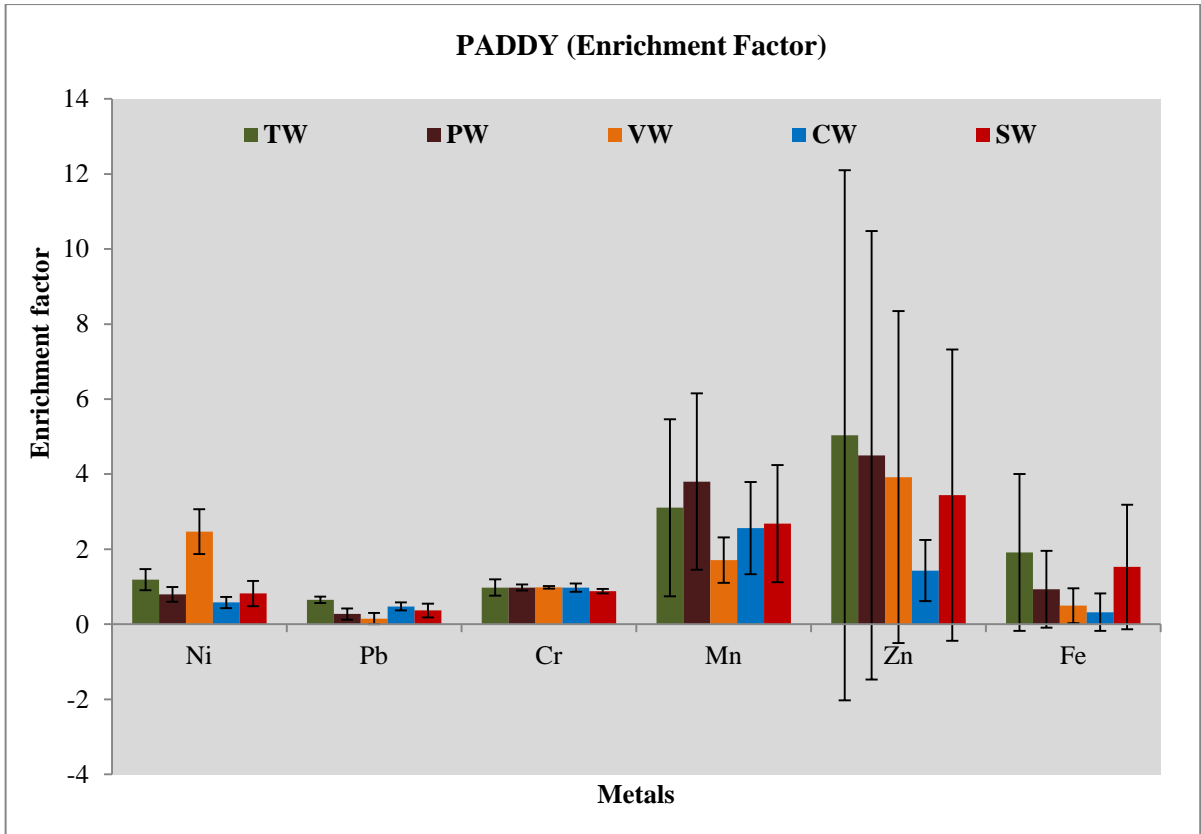


Figure 5.22: (a) Paddy (Enrichment factor) and (b) Wheat (Enrichment Factor)

## RESEARCH PAPER-III

### To assess comprehensive risk of wetland treated and untreated sewage water use on the consumer health

---

#### Abstract

Food-chain transfer is the primary route of human exposure to environmental pollutants. Cereals have a higher contribution in the diet yet most wastewater risk or impact assessment studies have largely been focussed on the vegetable crops. Thus with this in view, the main aim of this investigation was to assess comprehensive metal risks due to the consumption of wheat and paddy food grains produced through wetland treated and untreated sewage waters. The analysis showed that overall metal health hazard, as evident from Hazard Index, due to the consumption of wheat grains produced through treated/ untreated wastewaters or ground waters on the historically metal contaminated soils was about 1.6 times more than that due to the consumption of paddy grains. About 45 to 60% of these health hazards were contributed by lead contamination. In general, food grains produced through (lead-sequestering) *Phragmites karka* treated wastewaters were observed to be associated with 44 to 58% less health hazards. Thus, from health point of view, the agricultural produce from the sewage plot sites was still not suitable for human consumption; especially due to considerable food grain metal viz., lead >> iron > nickel ~ Mn contamination. However these risks were far lower than those during previous year, primarily due to continuous application of wetland treated sewage waters and thus gradual reduction of soil bio-available and total metal concentrations on the historically sewage water irrigated soils.

#### 6.1 Introduction

Food and water security for rapidly increasing population is day by day becoming a global challenge. This is very evident from the basic fact that due to rapid population growth (80 million or 1.2% per annum) water withdrawals have tripled over last 50 years and the potential global water availability has decline from 12,900 m<sup>3</sup> per capita per year in 1970 to 7,000 m<sup>3</sup>, in 2000.

Agriculture is the largest user of water, accounting for nearly 70% of all worldwide extractions (Gleick, 2000; FAO, 2008). In developing countries due to receding water levels and due to lack of suitable wastewater treatment facilities, use of unplanned and untreated/ improperly treated sewage waters is on an increase. In fact,

globally, re-use of marginal quality waters, with or without any treatment, in agriculture is being viewed as one of the practical wastewater management options.

At present, at least one-tenth of the world's population consumes crops irrigated with untreated or poorly treated wastewater, mostly in developing countries in Africa, Asia and Latin America (Smith & Nasr, 1992) as it fulfils the need of irrigation water to resource poor farmers. However, among the hazards associated with the wastewater use in agriculture, food contamination due to heavy metals like lead, cadmium, mercury, nickel etc. are the primary ones. More than ten thousands of toxic chemicals from different industries, agricultural chemicals and household products are routinely used and released in the wastewater and drains into sanitary sewers which can create hazard to different animals including human. Some chemicals derived from pesticides, persistent organic pollutants, non-ionic detergents and human and veterinary pharmaceutical residues are carcinogenic, teratogenic and mutagenic in nature and also act as endocrine disrupters, which mimic hormones and have antihormonal activity and so interfere with the functioning of endocrine systems in various species. These substances are resistant to conventional wastewater treatment and so persist in environment for some times (National Research Council, 1998) and can cause breast, prostate and testicular cancers, impaired behavioural/mental, immune and thyroid functions in children. Reproductive abnormalities, altered immune function and population disruption have been observed in amphibians, birds, fishes, invertebrates, reptiles and mammals.

Some of the harmful impacts of intake of toxic metals become apparent only after several years of exposure (Bahemuka and Mubofu, 1999; Ikeda et al., 2000). Some studies have reported that consumption of heavy metal contaminated food can deplete some essential nutrients in the body that are further responsible for decreasing immunological defences, intrauterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Iyengar and Nair, 2000; Tu'rkdogan et al., 2003).

Food-chain transfer is the primary route of human exposure to environmental pollutants i.e. transfers of pollutants via the wastewater → Soil → Plant → Human, in which pollutant intake from the consumption of grain, vegetables, root/tuber crops and fruit and can contaminate and affect entire food-chain. Pollutant concentration increases as we go higher level in the food chain due to bio-accumulation and bio-magnification. The higher organisms, mostly humans, occupy generally the top most

position in the trophic level in the food chain and so have chances of being impacted the most.

Environmental and human health risks due to wastewater agriculture/ use can be minimized by suitable treatment of wastewater before use, careful handling, wise application and proper awareness. However the available technologies, due to their high capital and maintenance costs, are unaffordable by the developing world. The processes involved in several conventional treatment systems, except stabilization ponds, are difficult and costly to operate in developing countries due to their requirements for high energy, skilled labour and installation/ operation and maintenance costs (Carr and Strauss, 2001). Thus the developing world is in the need of such non-conventional low cost and environmentally sustainable treatment technologies that have lower capital, maintenance and operational costs and could be operated by less skilled persons.

Non-conventional wastewater treatment methods include the use of low-cost systems such as on-farm ponds, sedimentation traps, bio sand-filters and constructed wetlands. Wetland systems have shown promise in achieving large reductions in heavy metals (Kadlec and Knight, 1996). However, scanning of literature reveals that most of the studies on the wetland treated waste waters have been largely focussed on either the evaluation of plant treatment efficiency/ capacity or on the system cost benefit analysis. Further, though cereals have a higher contribution in the diet yet most wastewater risk or impact assessment studies have been largely focussed on the vegetable crops. Thus with this in view, the main aim of this investigation was to assess comprehensive risk of heavy metal contamination due to the consumption of cereals produced through wetland treated and untreated sewage waters.

## **6.2 Material and methods**

In order to achieve the set objectives, a pilot scale vertical sub-surface flow (VSSF) constructed wetland system was developed and sited near a domestic sewage water drain on a 350 m<sup>2</sup> land area (28°38'21.3" N and 77°08'56.5" E) within the Seed Production Unit (SPU) farm of the Indian Agricultural Research Institute (IARI). Sewage water generated from the IARI micro watershed, containing moderate organic load, lab chemical compounds and heavy metals, was bypassed through the aforementioned system, treated and thereafter used for irrigating Wheat and Paddy crops in the adjacent 18- micro-plots. The details on the treatment wetlands and the

experimental micro-plot layout and crops/ cultural practices are illustrated in sections 3.2.1 and 3.2.2.

For analysing the impact of water treatments on the produce quality, plant samples were collected. In case of wheat, four plants each of three height groups viz. long (Main), medium (Mid) & short (Low) of total twelve healthy plants and in case of paddy, three tillers of three height groups viz. long (Main), medium (Mid) & short (Lower) of total five healthy plants per each micro-plot were randomly tagged, just after grain filling stage, and used for analysis. These tagged plants having intact root were sampled with khurpi just before harvesting of the rest of the plants. Each micro-plot plant sample was bundled and immediately transferred to laboratory and air dried. Root and shoot parts were oven dried (at 55°C) and ground (individually) along with grains through a milling machine and subjected to micro-nutrient (viz. Zn, Cu, Fe, Mn) and trace metal (viz. Cd, Cr, Co, Ni, Pb) analysis as per the procedure detailed in section (3.3.2.3). All samples were analysed in triplicate; with due quality control ensured through careful standardization, procedural blank measurements and duplicate samples in the Hydro-informatics and Wetland bio-geochemistry laboratories of the Division of Environmental Sciences, IARI.

Finally the threat of heavy metal contamination, due to untreated and treated waste water irrigations, on the consumer (i.e. human) health was assessed in terms of the **Health Risk Index (HRI)** as per the following equation:

$$\text{Health Risk Index (HRI)} = \text{HQ} \times f$$

where,  $f$  is the consumption probability of contaminated grains and HQ is Hazard Quotient. Pierzynski et al. (2000) evaluated the Hazard Quotient in the following manner:

$$\text{HQ} = (\text{Dietary intake of a specific heavy metal}) / (\text{Its Safe limit})$$

where, Dietary intake of heavy metal (in mg metal/Kg body weight/day) is generally obtained as (Concentration of metal in contaminated food  $\times$  Daily food intake) / (Average body weight). HRI index value  $>1$  is considered unsafe for human health (USEPA 2002).

The composite risk due to the consumption of heavy metal contaminated grains was also analyzed in terms of a **Hazard Index** (Huang et al. 2007), which is the measure of the potential risk of adverse health effects from a mixture of heavy metal constituents in the contaminated grains. When the hazard index exceeds 1.0 there is concern for potential health effects.

$$HI = \sum_{i=1}^n HRI$$

For this, the daily food intake pattern of the exposed individual was represented by the global diet (Galal-Gorchev, 1991) which considers daily intake of grains/cereals of about 0.405 kg (on fresh weight basis where the fresh weight to dry weight conversion factor is 0.90 for grains/cereals). The dietary intake of pollutants normally does not exceed 50% of the Provisional Tolerable Weekly Intake (PTWI), except for individuals who are exposed through occupational activities or are resident near a pollution point source (WHO, 1998; USEPA, 1992a & 2000). Hence for this analysis, the consumption probability of contaminated grains was assumed as 25%. Further, the average body weight of an adult was considered as 70kg while the oral reference dose or safe daily intake quantity of different heavy metals, as per USEPA (1997 & 2002), was considered to be Cu (0.04mg/kg/day), Zn (0.3 mg/kg/day), Cd (0.001 mg/kg/day), Pb (0.004 mg/kg/day), Ni (0.02 mg/kg/day), Cr (1.5 mg/kg/day), Fe (0.25 mg/kg/day) and Mn (0.14 mg/kg/day), Co (1.6 mg/kg; FSA, 2003).

### 5.3 Results and Discussions

The analysis showed that though chromium was the key contaminant in the treated/ untreated wastewaters (Figure 4.10; section-4) and was also present beyond permissible levels (in both bio-available and total forms, Figure 4.13b and 4.15b; section-4) in the historically sewage water irrigated experimental soils. Yet its dietary intake through wheat and paddy grains (Table 6.1 and 6.2; Figure 6.1, 6.2 and 6.3) was well within the oral reference dose (1.5 mg/kg/day) thereby leading to only 2 – 5% health risk. This was also found to be the case with cobalt contamination, whose dietary intake was just 1.7 % of its reference oral dose (Figure 6.2d) through wheat grains and 0% through paddy grains.

Dietary intakes of micro-nutrients viz. manganese (Mn; Figure 6.1d) and zinc (Zn; Figure 6.1e), through paddy grains were also observed to be well within safe limits and associated with less health risks (i.e. 0.10 and 0.07, respectively) due to food produced with both treated and untreated wastewaters. However, though the dietary intake of Zn through wheat grains was somewhat within safe limits, the dietary intake of Mn through wheat grains appeared to be about 2.8 and 1.4 times more than its prescribed safe limit for the food produced through untreated/ treated wastewaters and that produced through the groundwater, respectively in the native manganese contaminated soils (Figure 4.14a; section-4). Thus, consumption of

treated/ untreated sewage water produced wheat grains was observed to be associated with about 6.4 times larger Mn-health risk than those through the paddy grains. Even the ground water produced wheat grains were observed to be associated with about 4.1 times higher Mn-health risk than that through paddy grains.

Dietary intake of nickel (Ni) through wheat grains (Figure 6.2a) also appeared to be about 2.8 times more than its prescribed safe limit for the food produced through untreated/ treated wastewaters or groundwater and thus was associated with about 2.4 times more health risk (HRI=0.70) than that through the consumption of paddy grains (HRI = 0.29). However Ni - health risks due to the consumption of both wheat and paddy grains were well within 1 and thus very low to low. Similar results were also observed with respect to iron (Figure 6.4a and 6.4b).

Table 6.1: Dietary Intake (DI) of various metals through wheat (mg/kg/day)

<b>METAL</b>	<b>TW</b>	<b>PW</b>	<b>VW</b>	<b>CW</b>	<b>SW</b>	<b>GW</b>	<b>ORD</b>
<b>Ni</b>	0.065 ± 0.008	0.055 ± 0.002	0.051 ± 0.008	0.059 ± 0.006	0.053 ± 0.001	0.051 ± 0.002	0.02
<b>Pb</b>	0.086 ± 0.006	0.032 ± 0.019	0.059 ± 0.040	0.083 ± 0.025	0.071 ± 0.034	0.081 ± 0.005	0.004
<b>Cr</b>	0.32 ± 0.05	0.27 ± 0.01	0.26 ± 0.00	0.31 ± 0.06	0.33 ± 0.05	0.31 ± 0.05	1.5
<b>Mn</b>	0.33 ± 0.02	0.37 ± 0.23	0.34 ± 0.21	0.57 ± 0.21	0.34 ± 0.20	0.19 ± 0.05	0.14
<b>Zn</b>	0.288 ± 0.006	0.299 ± 0.049	0.299 ± 0.006	0.267 ± 0.043	0.285 ± 0.033	0.281 ± 0.031	0.3
<b>Fe</b>	1.24 ± 0.80	0.68 ± 0.56	1.09 ± 0.89	0.47 ± 0.25	0.56 ± 0.20	0.23 ± 0.12	0.25
<b>Co</b>	0.028 ± 0.001	0.026 ± 0.001	0.026 ± 0.001	0.028 ± 0.004	0.026 ± 0.00	0.026 ± 0.00	1.6

Table 6.2: Dietary Intake of various metals through paddy (mg/kg/day)

<b>METAL</b>	<b>TW</b>	<b>PW</b>	<b>VW</b>	<b>CW</b>	<b>SW</b>	<b>GW</b>	<b>ORD</b>
<b>Ni</b>	0.028 ± 0.004	0.022 ± 0.004	0.030 ± 0.008	0.022 ± 0.010	0.018 ± 0.002	0.021 ± 0.005	0.02
<b>Pb</b>	0.080 ± 0.008	0.021 ± 0.009	0.015 ± 0.015	0.065 ± 0.020	0.047 ± 0.020	0.085 ± 0.008	0.004
<b>Cr</b>	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	1.5
<b>Mn</b>	0.051 ± 0.010	0.075 ± 0.018	0.047 ± 0.022	0.05 ± 0.0147	0.06 ± 0.008	0.04 ± 0.007	0.14
<b>Zn</b>	0.108 ± 0.038	0.087 ± 0.040	0.108 ± 0.058	0.05 ± 0.0194	0.099 ± 0.018	0.048 ± 0.032	0.3
<b>Fe</b>	0.59 ± 0.28	0.29 ± 0.16	0.24 ± 0.33	0.16 ± 0.27	0.46 ± 0.27	0.45 ± 0.26	0.25

Health risks due to higher dietary intake of lead (Pb, Figure 6.4a and 6.4b) appeared to be of key concern as these appeared to be about 13 to 17 times more than their permissible safe limits for both wheat and paddy grains. In general the food grains produced from *Phragmites* treated wastewaters were observed to be associated with far lower health risks (1.29 to 1.97). These were found to be just 29-39% of those through other irrigation waters (4.34 to 5.02, Figure 6.4a and 6.4b).

Thus overall metal health hazard, as evident from Hazard Index, due to the consumption of wheat grains produced through treated/ untreated wastewaters or ground waters (Table 6.5 and Figure 6.5) on the historically metal contaminated soils was about 1.6 times more than that due to the consumption of paddy grains. About 45 to 60% of these health hazards were observed to be contributed by lead contamination. In general, food grains produced through (lead-sequestering) *Phragmites karka* treated wastewaters were observed to be associated with 44 to 58% less health hazards. Thus, from health point of view, the agricultural produce was still not suitable for human consumption especially due to high lead contamination in both paddy and wheat grains. However these risks were far lower than those during previous year (Table 6.5; Figure 6.6a and 6.6b), primarily due to continuous application of wetland treated sewage waters and thus gradual reduction of soil bio-available and total metal concentrations (Figure 4.12, 4.13, 4.14 and 4.15) on the historically sewage water irrigated and metal contaminated soils.

Table 6.3: Health Risk Index (HRI) by different metals intake through paddy in an adult human

SAMPLE	Ni	Pb	Cr	Mn	Zn	Fe
<b>TW</b>	0.35 ± 0.05	5.02 ± 0.52	0.02 ± 0.00	0.09 ± 0.02	0.09 ± 0.03	0.59 ± 0.28
<b>PW</b>	0.27 ± 0.05	1.29 ± 0.57	0.02 ± 0.00	0.13 ± 0.03	0.07 ± 0.03	0.29 ± 0.16
<b>VW</b>	0.37 ± 0.10	0.94 ± 0.94	0.02 ± 0.00	0.08 ± 0.04	0.09 ± 0.05	0.24 ± 0.33
<b>CW</b>	0.27 ± 0.12	4.08 ± 1.26	0.02 ± 0.00	0.10 ± 0.02	0.05 ± 0.02	0.16 ± 0.27
<b>SW</b>	0.23 ± 0.02	2.92 ± 1.25	0.02 ± 0.00	0.12 ± 0.01	0.08 ± 0.01	0.46 ± 0.27
<b>GW</b>	0.26 ± 0.07	5.33 ± 0.50	0.02 ± 0.00	0.08 ± 0.01	0.04 ± 0.03	0.45 ± 0.26

Table 6.4: Health Risk Index (HRI) by different metals intake through wheat in an adult human

SAMPLE	Ni	Pb	Cr	Mn	Zn	Fe
<b>TW</b>	0.82 ± 0.10	5.21 ± 0.43	0.05 ± 0.01	0.59 ± 0.04	0.24 ± 0.01	1.24 ± 0.80
<b>PW</b>	0.69 ± 0.02	1.97 ± 1.20	0.05 ± 0.00	0.66 ± 0.41	0.25 ± 0.04	0.68 ± 0.56
<b>VW</b>	0.64 ± 0.11	3.71 ± 2.51	0.04 ± 0.00	0.60 ± 0.37	0.25 ± 0.01	1.09 ± 0.89
<b>CW</b>	0.74 ± 0.07	5.21 ± 1.55	0.05 ± 0.01	1.02 ± 0.37	0.22 ± 0.04	0.47 ± 0.25
<b>SW</b>	0.67 ± 0.02	5.88 ± 0.27	0.06 ± 0.01	0.61 ± 0.36	0.24 ± 0.03	0.56 ± 0.20
<b>GW</b>	0.63 ± 0.03	5.08 ± 0.30	0.05 ± 0.01	0.33 ± 0.38	0.23 ± 0.03	0.23 ± 0.12

Table 6.5: Hazard Index (HI) of various metals intake through paddy and wheat in an adult human

SAMPLE	Wheat		Paddy	
	Season-I	Season-II	Season-I	Season-II
<b>TW</b>	13.28 ± 2.57	8.15 ± 1.38	9.93 ± 2.06	6.16 ± 0.27
<b>PW</b>	11.09 ± 5.66	4.30 ± 2.23	13.10 ± 8.95	2.08 ± 0.68
<b>VW</b>	26.50 ± 7.36	6.33 ± 3.89	7.03 ± 0.65	2.84 ± 0.59
<b>CW</b>	16.62 ± 7.49	7.72 ± 2.29	11.95 ± 3.31	4.68 ± 1.58
<b>SW</b>	13.43 ± 6.81	8.02 ± 0.88	50.20 ± 26.12	3.84 ± 1.55
<b>GW</b>		6.57 ± 0.56	10.88 ± 0.99	6.19 ± 0.81

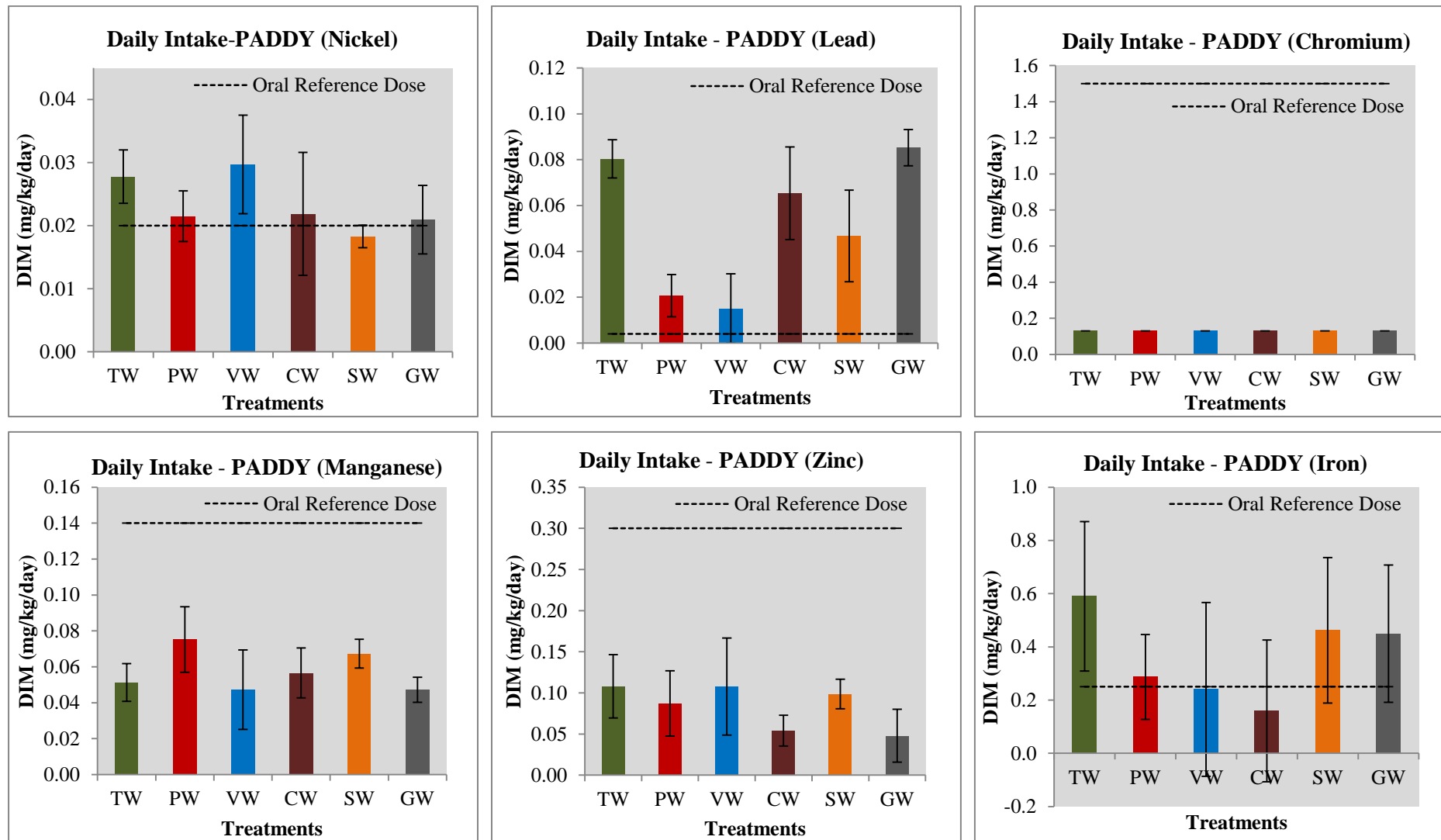


Figure 6.1: Daily intake of various metals (a) Nickel, (b) Lead, (c) Chromium, (d) Manganese, (e) Zinc and (f) Iron through Paddy grains grown on different irrigation plots along with their oral ref. dose

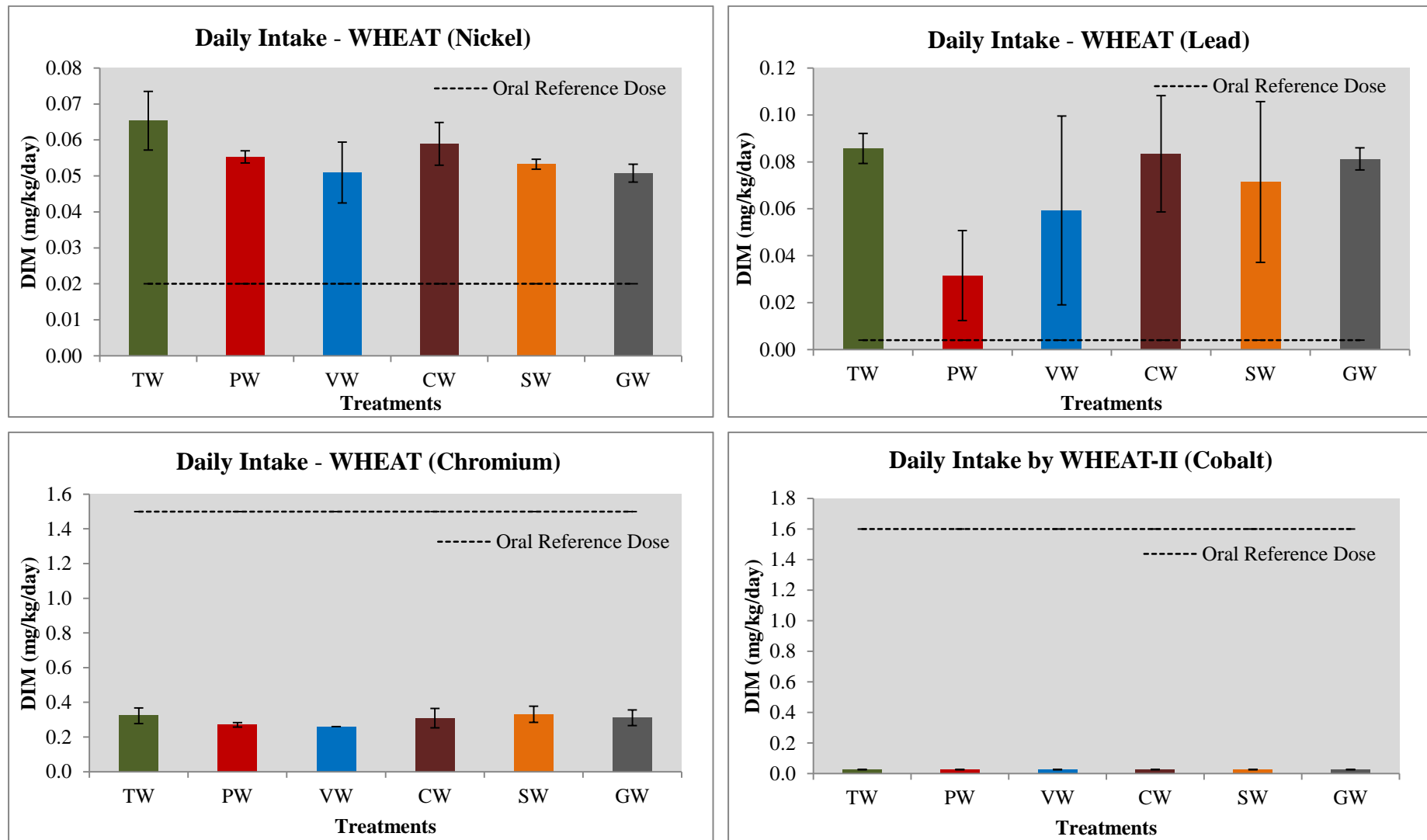


Figure 6.2: Daily intake of various metals (a) Nickel, (b) Lead, (c) Chromium and (d) Cobalt through Wheat grains grown on different irrigation plots along with their oral reference dose

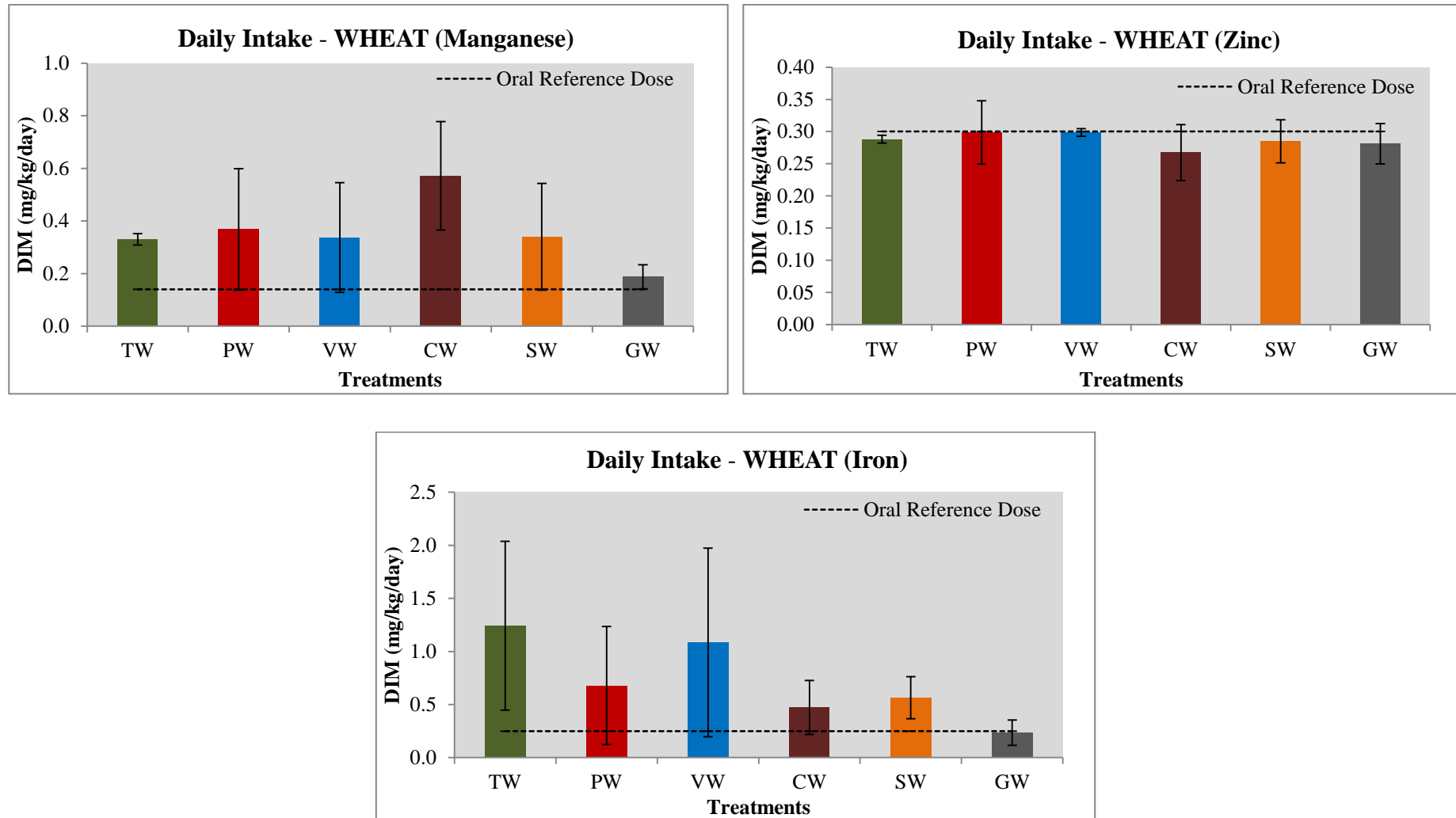


Figure 6.3: Daily intake of various metals (a) Manganese, (b) Zinc and (c) Iron through Wheat grains grown on different irrig plots along with their oral Reference dose

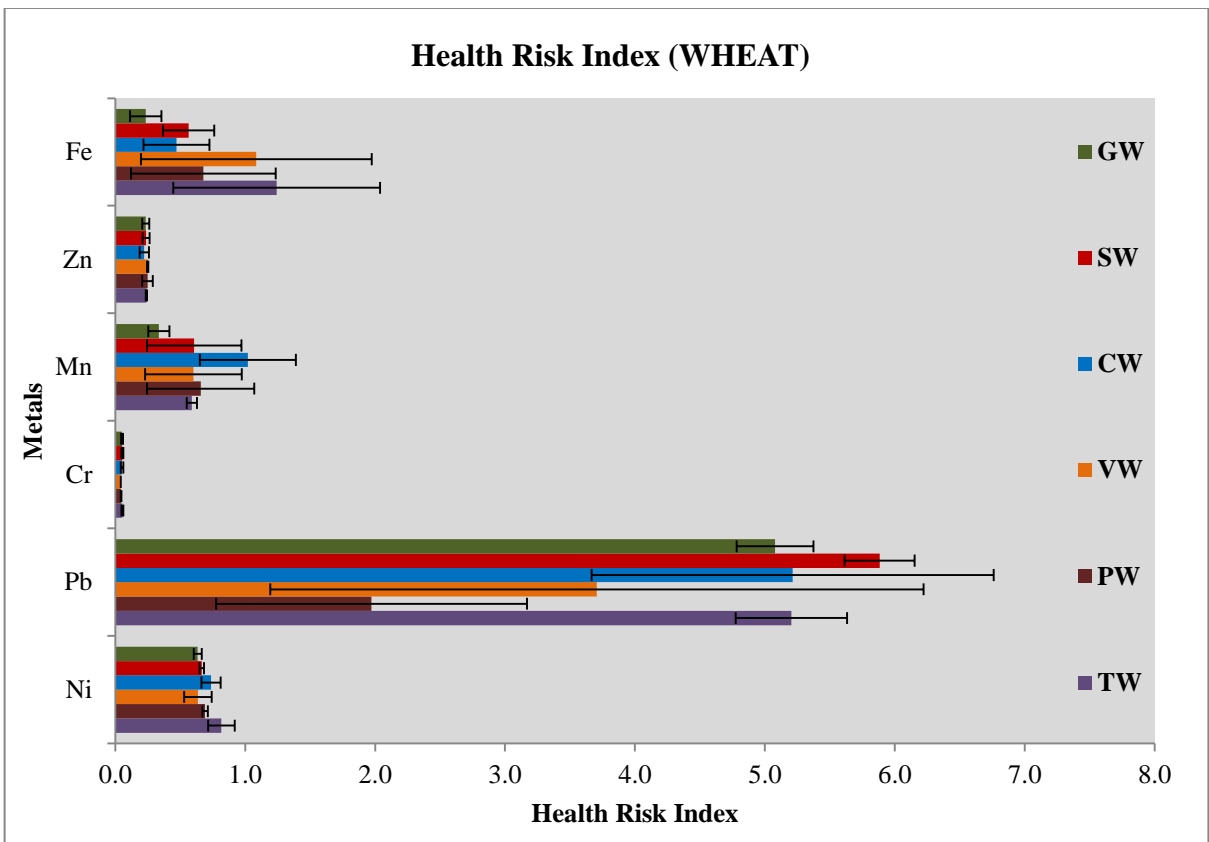
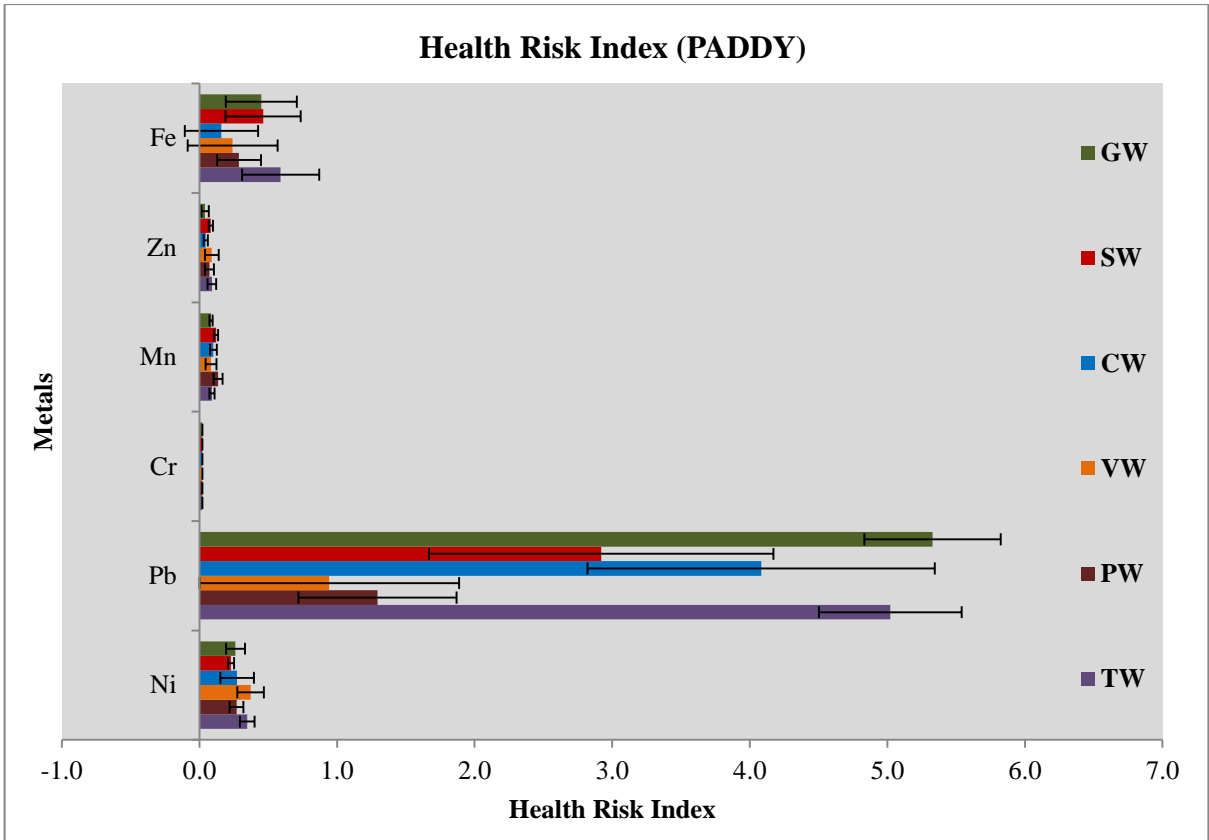


Figure 6.4: Health risk Index (HRI) of (a) Paddy and (b) Wheat

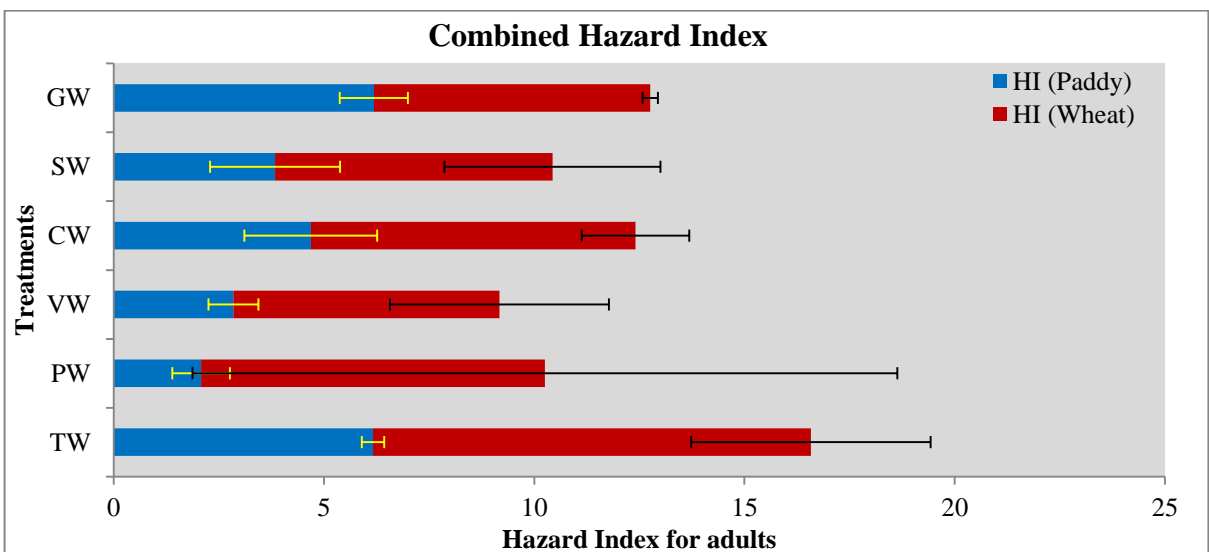
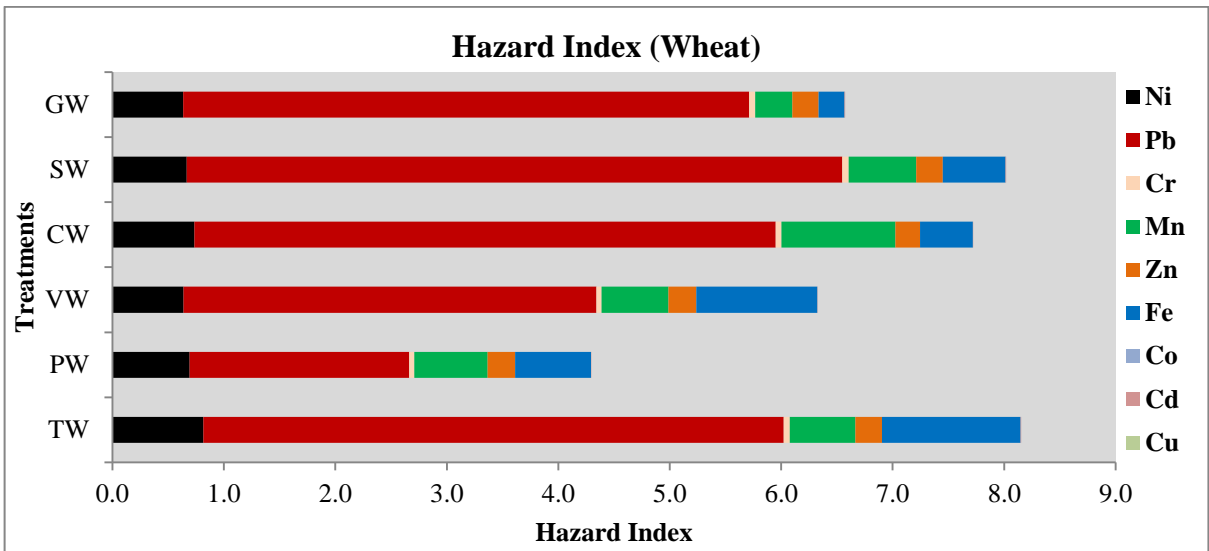
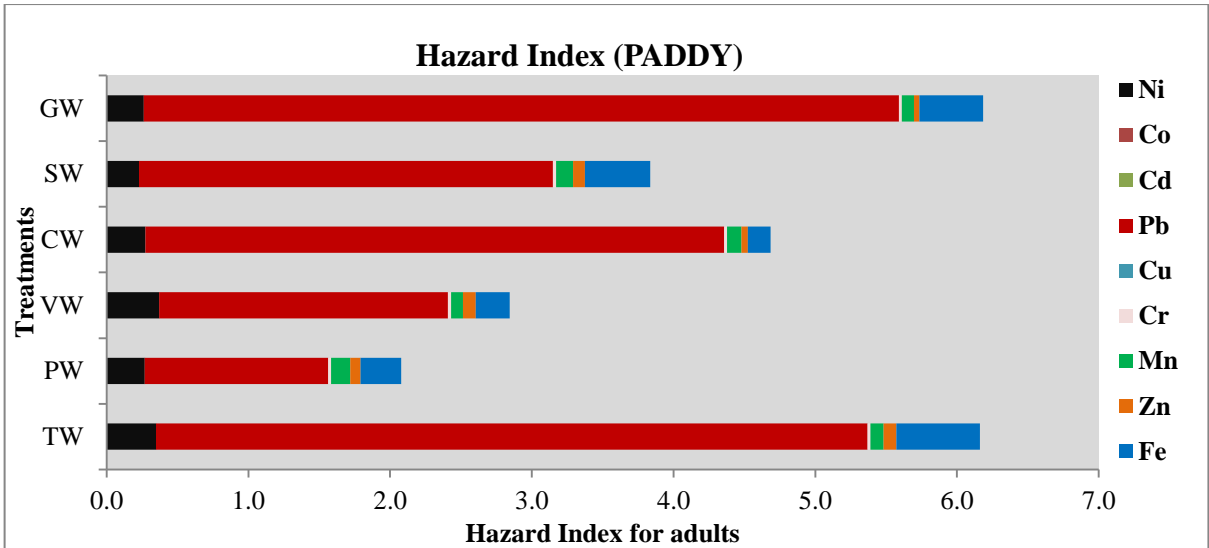


Figure 6.5: Hazard Index of (a) Paddy, (b) Wheat and (c) Combined wheat and paddy crop

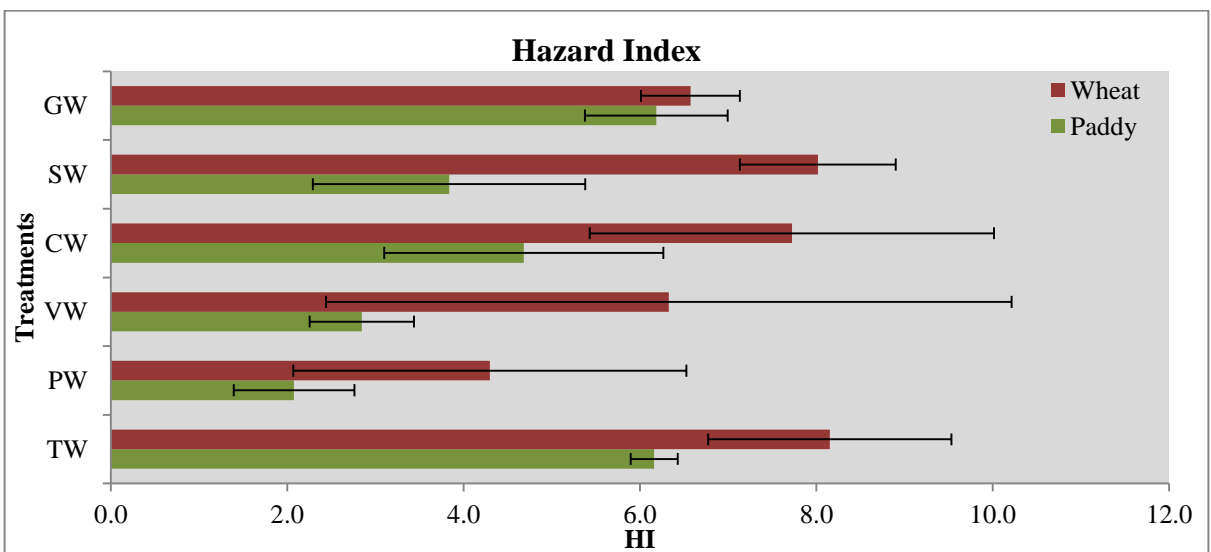
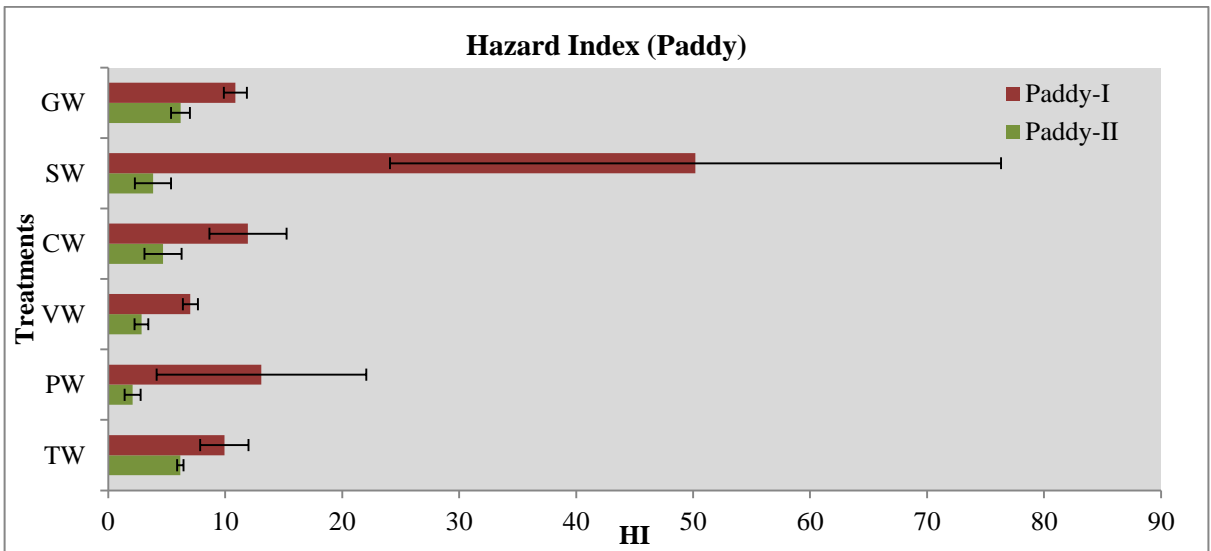
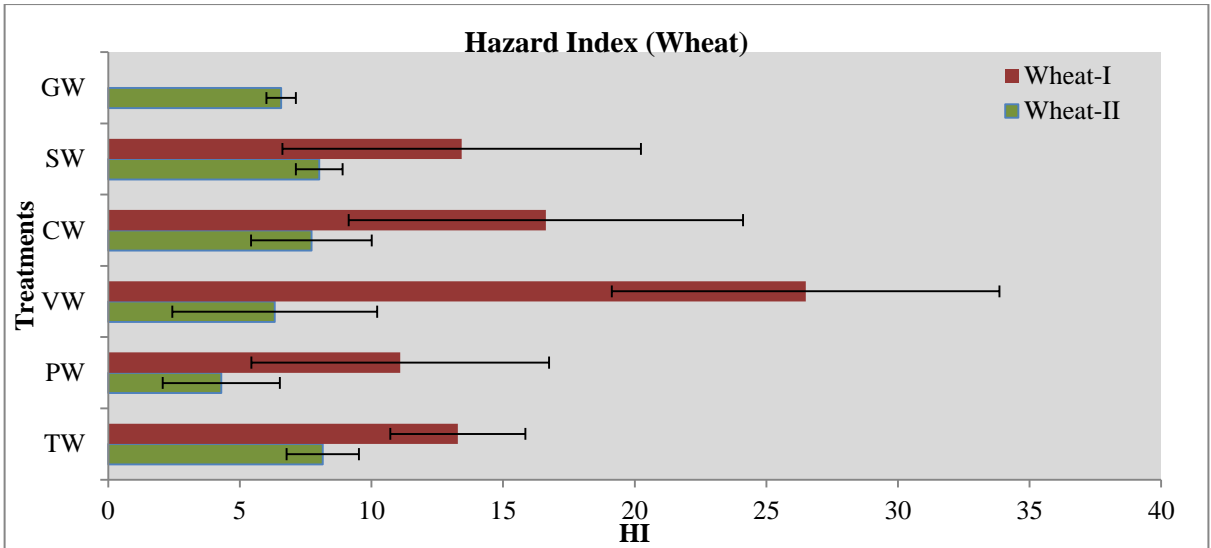


Figure 6.6: (a) Hazard Index (Wheat), (b) Hazard Index (Paddy) and Comparative Hazard Index (Wheat- Paddy)

## SUMMARY AND CONCLUSION

---

Freshwater scarcity, generation of increasing volumes of wastewaters, degradation of freshwater resources and the interconnected food insecurity, due to rapid urbanization/ industrialization, is driving many countries to use marginal quality waters in agriculture. Agricultural reuse of sewage wastewater is fast becoming popular worldwide because it closes the loop between water demand and wastewater disposal and enhances fertilizer security of resource poor farmers. However, due to the lack of proper treatment facilities and awareness in developing countries, unplanned application of raw sewage is increasing the risk of agricultural sustainability and consumer/ environmental health. Thus for safe agricultural disposal, with optimum profit, the scope of harvesting low cost, low energy optimum level treatment options is increasing. Among different low cost/ low energy treatment options, use of treatment wetlands is on increase. However, scanning of literature reveals that most of such existing studies lack comparative comprehensive risk of untreated and treated sewage water use on the soil, crop and consumer health. Further, though cereals have a higher contribution in the diet, yet most of such risk or impact assessment studies have primarily been focussed on the vegetable crops. One such wetland technology based pilot sewage water treatment plant (of 1500 LPD capacity) is in operation since November 2009, at the Indian Agricultural Research Institute (IARI). Hence, the main goal of the present investigation was to assess an overall impact of the wetland treated wastewaters on the quality of agricultural soil and produce and thus risk to human health.

In order to achieve this goal 18- micro-plots (each of 2.7 m<sup>2</sup> area), under Rice-Wheat cropping system, were continuously irrigated with 4 types of subsurface flow constructed wetland treated and untreated sewage waters at the sewage plot site of IARI (New Delhi) for two years. Routine crop specific management practices were applied and suitable samples of irrigation waters, soils and plants were periodically analyzed for different nutrient and metal concentrations. Food-chain contamination factors and suitable risk indices were also calculated for assessing comprehensive risk of treated/ untreated wastewaters on the soil, plant and consumer health.

During the study period, temperature, pH and dissolved oxygen levels of the untreated sewage waters were observed to be within the permissible limits (i.e. 30°C, 8.0 and 5mg/l) while turbidity ( $67.01 \pm 42.57$  NTU) was found to be mostly beyond

permissible level (50 NTU). In comparison to these, the wetland treated sewage waters were associated with significantly (40 to 74%) lower turbidity (17.70 NTU), nitrate (2.78 ppm), phosphate (11.75 ppm) and potassium (9.39 ppm) levels. Sulphate, fluoride, EC and TDS levels of the irrigation waters were also in general found to be within the permissible limits. Amongst heavy metals, chromium, manganese and iron were the key contaminants that were present in their more than permissible levels. Rest metals viz. nickel, lead, zinc, though present in appreciable amounts were within their safe limits.

Continuous application of treated sewage waters, for 2-years, in place of untreated sewage water applications at the project site resulted in significant reduction in soil potassium concentration from an initial (beyond permissible) levels of  $(219.19 \pm 12.66 \text{ mg/kg})$  to  $(125.27 \pm 10.57 \text{ mg/kg})$ . Soil nitrate and phosphate concentrations also decreased by about 88% and 38%, respectively thereby showing a significant impact of water treatment on the soil nutrient load. Soil total and bio-available nickel (Ni), lead (Pb), and iron (Fe) concentrations also decreased significantly. Soil bio-available chromium decreased from an initial level of  $(5.71 \pm 0.88 \text{ mg/kg})$  to  $(1.57 \pm 0.07 \text{ mg/kg})$  after two years. However, there was no change in the soil total chromium (Cr) level, due to its more than (1.7 times) permissible concentrations in the historically sewage water irrigated soils of the experimental area. Soil bio-available zinc concentrations changed insignificantly from  $(6.38 \pm 0.55 \text{ mg/kg})$  to  $(5.81 \pm 0.51 \text{ mg/kg})$  and cadmium and copper concentrations were found to be below detection limits throughout the research period. Thus, continuous irrigation with treated sewage waters led to significant reductions in soil pollutant load. However these were associated with no soil micro-nutrient depletion and any adverse effects due to soil electrical conductivity and exchangeable sodium percentage, which remained within safe limits.

The impact of the untreated and treated sewage waters on the health and quality of the Wheat and Paddy crops was also estimated in terms of plant/ seed parameters, individual metal translocation pattern and food grain metal sequestration threat. The positive impact of water treatment could be best expressed in terms of the test weight or 100 seed weight of the Paddy crop, as it was found to be significantly lower for the wastewater irrigated crop. Further, though total number of tillers and length of panicle were not significantly different for the treated and the untreated

sewage waters, yet, total number of unproductive tillers and the unfilled seeds per panicle were significantly higher in the sewage water irrigated paddy crop. These differences were not very evident in the relatively low water demanding crop i.e. wheat seeds produced from treated/ untreated wastewaters. However, number of termite and fungal infected tillers in both wheat and paddy were observed to be higher in the sewage water irrigated micro-plots. Individual metal translocation patterns in the wheat and paddy crop plants revealed higher food grain metal sequestration threat in wheat. In general, wheat grains produced with treated wastewater discharged from wetlands containing *Phragmites karka* and *Acorus calamus* were observed to be sequestering significantly lower lead and nickel/ cadmium concentrations, respectively than those produced with the untreated sewage waters. Similarly paddy grains produced with treated sewage waters discharged from the wetlands containing *Phragmites karka* and *Acorus calamus*/ unplanted mesocosm seemed to be sequestering significantly lower cadmium and iron, respectively.

The analysis showed that the overall metal health hazard (as evident from Hazard Index) due to the consumption of wheat grains produced through treated/ untreated wastewaters or ground waters on the historically metal contaminated soils was about 1.6 times more than that due to the consumption of paddy grains. About 45 to 60% of these health hazards were contributed by lead contamination. In general, food grains produced through (lead-sequestering) *Phragmites karka* treated wastewaters were observed to be associated with 44 to 58% less health hazards. From health point of view, the agricultural produce from the sewage plot sites was still not suitable for human consumption; especially due to considerable food grain metal viz., lead >> iron > nickel ~ Mn contamination. However these risks were far lower than those during previous year, primarily due to continuous application of wetland treated sewage waters and thus gradual reduction of soil bio-available and total metal concentrations on the historically sewage water irrigated soils. Thus, the analysis could clearly re-confirm the need for proper treatment and scientific application of the poor quality irrigation waters for maintaining sustainable land / food qualities.

## Impact of wetland-treated sewage water applications on the soil health and agricultural produce from the experimental sewage plot site of Indian Agricultural Research Institute

---

### Abstract

A comparative impact of experimental subsurface flow constructed wetland treated and the raw (untreated) sewage water applications under Paddy-Wheat cropping sequence was evaluated on the historically sewage water contaminated experimental plots of Indian Agricultural Research Institute. Continuous application of treated sewage waters, for 2-years, in place of untreated sewage water applications (i.e. *business-as-usual*) at the project site resulted in significant reduction in soil potassium concentration from an initial (beyond permissible) level of  $(219.19 \pm 12.66 \text{ mg/kg})$  to  $(125.27 \pm 10.57 \text{ mg/kg})$ . Soil nitrate and phosphate concentrations also decreased by about 88% and 38%, respectively thereby showing a significant impact of water treatment on soil nutrient load. Soil total and bio-available nickel (Ni), lead (Pb), chromium (Cr) and iron (Fe) concentrations also decreased significantly. However, continuous irrigation of treated sewage waters lead to no depletion of soil micro-nutrient concentrations as well as any adverse effects due to soil electrical conductivity and exchangeable sodium percentage, which remained within safe limits. Wheat and Paddy crops irrigated with treated sewage waters were observed to be associated with larger 1000 seed weights, lower number of unfilled unproductive tillers and the unfilled seeds per panicle and termite and fungal infected tillers. Wheat and paddy grains produced with *Phragmites karka* and *Acorus calamus* treated wastewater were observed to be sequestering significantly lower lead, nickel and cadmium, iron, respectively. Thus food grains produced through *Phragmites karka* treated wastewaters were observed to be associated with 44 to 58% less health hazards. Though, from health point of view, the agricultural produce from the sewage plot sites was still not suitable for human consumption; especially due to considerable food grain metal viz., lead  $\gg$  iron  $>$  nickel  $\sim$  Mn contamination. Yet these risks were far lower than those during previous year, primarily due to continuous application of wetland treated sewage waters and gradual reduction of soil bio-available and total metal concentrations on the historically sewage water irrigated soils. Thus, compared to the untreated sewage waters, the wetland treated sewage waters seemed to be associated with considerably lower environmental foot-print.

**भारतीय कृषि अनुसन्धान संस्थान के सीवेज प्रदूषित भूमि पर आर्द्रभूमि द्वारा साफ किये गए सीवेज पानी के अनुप्रयोगों का उसकी मिट्टी के स्वास्थ्य एवं कृषि उपज पर प्रभाव**

**सार**

धान - गेहूँ की फसल अनुक्रम के तहत प्रयोगात्मक उपसतह प्रवाह आर्द्रभूमि द्वारा उपचारित सीवेज पानी एवं कच्चे मलजल (अनुपचारित सीवेज) पानी के अनुप्रयोगों का तुलनात्मक प्रभाव ऐतिहासिक सीवेज पानी दूषित भारतीय कृषि अनुसंधान संस्थान की प्रायोगिक भूखंडों पर मूल्यांकन किया गया। निरंतर दो साल के लिए उपचारित सीवेज पानी के अनुप्रयोगों से परियोजना स्थल (व्यापार के रूप में हमेशा की तरह) की मिट्टी के पोटेशियम सांद्रता में प्रारंभिक (परे) अनुमेय स्तर  $219.19 \pm 12.66$  मिलीग्राम प्रति किलोग्राम से  $125.27 \pm 10.57$  मिलीग्राम प्रति किलोग्राम तक की एवं नाइट्रेट और फास्फेट सांद्रता में 88% और 38% की महत्वपूर्ण कमी हुई। जिससे क्रमशः मिट्टी में पोषक तत्वों की सांद्रता पर जल उपचार के महत्वपूर्ण प्रभाव दिखा। मिट्टी के धातु (जैव उपलब्ध एवं कुल) निकल, सीसा, क्रोमियम और लोहा की सांद्रता में भी काफी कमी आई है। हालांकि, उपचारित सीवेज पानी की निरंतर सिंचाई से मिट्टी के सूक्ष्म पोषक तत्वों की सांद्रता में एवं मिट्टी विद्युत चालकता और विनिमय सोडियम प्रतिशत के कारण कोई प्रतिकूल प्रभाव नहीं पाया गया; क्योंकि उनकी सांद्रता सुरक्षित सीमाओं के भीतर थीं। उपचारित सीवेज पानी से सिंचित फसलों (गेहूँ और धान) में प्रति हजार बीज का वजन ज्यादा, खोखले बीज व अनुत्पादक टिलर की संख्या और दीमक व कवक संक्रमित टिलर की संख्या कम पायी गयी। रीड (*फ्रैगमाइट्स कारका*) और बचा (*अकोरस कैलमस*) उपचारित सीवेज पानी से उत्पादित गेहूँ और धान के दानों में अनुपचारित सीवेज की अपेक्षा काफी कम सीसा, निकल, कैडमियम और लोहा पाया गया। इस प्रकार अनुपचारित सीवेज की अपेक्षा रीड उपचारित सीवेज पानी से उत्पादित अनाज 44-58% कम स्वास्थ्य के खतरों के साथ संबद्ध पाया गया। यद्यपि, स्वास्थ्य की दृष्टि से, सीवेज उपचारित प्रायोगिक भूखंडों से कृषि उपज अभी भी उपयुक्त नहीं है, विशेष रूप से अत्यधिक धातु (सीसा >> लोहा > निकल ~ मैंगनीज) संदूषण के कारण। वैसे अनुपचारित सीवेज के जगह निरंतर आर्द्रभूमि उपचारित सीवेज पानी से सिंचाई के कारण मिट्टी में धातु (जैव उपलब्ध एवं कुल) की सांद्रता का क्रमिक कमी की वजह से स्वास्थ्य के खतरों में पिछले वर्ष की अपेक्षा काफी कमी आई है। मुख्यतः. इस प्रकार, अनुपचारित सीवेज पानी की तुलना में, आर्द्रभूमि उपचारित सीवेज पानी को कम पर्यावरण प्रदूषण (फूट-प्रिंट) संबद्ध पाया गया।

## APPENDIX

Table 9.1: Recommended irrigation water quality parameters

Parameters	Safe limit	References
pH	6.0-8.0	WAPDA (1981)
	6.5-8.5	Acharya et al. (2008)
EC	2.25 dS/m	Acharya et al. (2008)
Temperature	30°C	WAPDA (1981)
Dissolved oxygen (DO)	5.0 mg/l	
Turbidity	50 NTU	
Residual sodium carbonate (RSC)	< 1.25 Excellent 1.2-2.5 Good > 2.5 Bad	USSL (1954)
Sodium adsorption ratio (SAR)	< 10 Excellent 10 – 18 Good 18 – 26 Medium > 26 Bad	CPCB
Total dissolved solid (TDS)	2000 mg/l	FAO (1985), Acharya et al. (2008)
Sodium (Na)	9.0 me/l	FAO (1985)
Bi-carbonate (HCO <sub>3</sub> <sup>-</sup> )	8.5 me/l	
Boron (B)	3.0 mg/l	
Chloride (Cl)	10 me/l	
Nitrate (NO <sub>3</sub> <sup>-</sup> )	30 mg/l	
Calcium & Magnesium (Ca + Mg)	6 me/l 10 me/l	Acharya et al. (2008) Khodapanah et al. (2009)
Phosphate (PO <sub>4</sub> <sup>3-</sup> )	50 mg/l	
Sulphate (SO <sub>4</sub> <sup>2-</sup> )	50 mg/l	
Potassium (K)	0.5 me/l	Acharya et al. (2008)
Fluoride (F <sup>-</sup> )	1.0 mg/l	National Academy of Sciences (1972) and Pratt (1972)
Intestinal nematode	≤ 1 arithmetic mean no. of eggs per litre	WHO (1989)
Faecal coliform	≤ 1000 geometric mean no. per 100 ml	

<b>Metals</b>	<b>Safe limit</b>	<b>References</b>
Aluminium (Al)	5.0 mg/l	National Academy of Sciences (1972) and Pratt (1972)
Arsenic (As)	0.10 mg/l	
Beryllium (Be)	0.10 mg/l	
Cadmium (Cd)	0.01 mg/l	
Chromium (Cr)	0.10 mg/l	
Cobalt (Co)	0.05 mg/l	
Copper (Cu)	0.20 mg/l	
Iron (Fe)	5.0 mg/l	
Lead (Pb)	5.0 mg/l	
Lithium (Li)	2.5 mg/l	
Manganese (Mn)	0.20 mg/l	
Molybdenum (Mo)	0.01 mg/l	
Nickel (Ni)	0.20 mg/l	
Selenium (Se)	0.02 mg/l	
Vanadium (C)	0.10 mg/l	
Zinc (Zn)	2.0 mg/l	

Table 9.2: Recommended safe limit of different parameters for soil

Parameters	Safe limit	References
pH	4 - 8.5	Alloway. B.J. (1990)
EC	4000 $\mu\text{S}/\text{cm}$	
Organic Matter	0.86 % w/w	
Phosphorus	>7 mg/kg	
Potassium	>80 mg/kg	
Arsenic (As)	8 mg/kg 30 mg/kg	WHO (2006) Alloway. B.J. (1990)
Antimony	36 mg/kg	WHO (2006)
Barium (Ba)	302 mg/kg	
Beryllium (Be)	0.2 mg/kg	
Boron (B)	1.7 mg/kg	
Molybdenum (Mo)	0.6 mg/kg	
Selenium (Se)	6 mg/kg	
Silver (Ag)	3 mg/kg	
Thallium	0.3 mg/kg	
Vanadium	47 mg/kg	
Cadmium (Cd)	1 mg/kg 4 mg/kg	
Chromium (Cr)	100 mg/kg 150 mg/kg	Alloway. B.J. (1990) European Union, 2002
Cobalt (Co)	0.5 mg/kg	Murtaza et al. (2008)
Copper (Cu)	100 mg/kg 140 mg/kg	Alloway. B.J. (1990) European Union (2002)
Iron (Fe)	5000 mg/kg	Stewart et al. (1974)
Lead (Pb)	84.0 mg/kg 500 mg/kg	WHO (2006) Alloway. B.J. (1990)
Manganese (Mn)	500 mg/kg	Alloway. B.J. (1990)
Mercury (Hg)	1 mg/kg 7 mg/kg	Alloway. B.J. (1990) WHO (2006)
Nickel (Ni)	20 mg/kg 75 mg/kg	Alloway. B.J. (1990) European Union (2002)
Zinc (Zn)	250 mg/kg 300 mg/kg	Alloway. B.J. (1990) European Union (2002)

Table 9.3: Recommended safe limit of different metals for agricultural produce (crop)

<b>Metals</b>	<b>Safe limit (mg/kg)</b>	<b>References</b>
Cadmium (Cd)	5.0	Macnicol and Beckett (1985)
	0.2	Lee and Lee (2001, Republic of Korea)
	0.1	Kent and Everes, 1990; Ministry of Health of China (2005)
	0.05	Walker (1988)
Chromium (Cr)	1.0	Ministry of Health of China (2005)
Cobalt (Co)	1.0	
Copper (Cu)	40	FAO/WHO standard (Codex Alimentarius Commission, 1984)
	10	Ministry of Health of China (2005)
Iron (Fe)	46	
Lead (Pb)	2.5	Indian standard (Awashthi, 2000)
	0.4	Ministry of Health of China (2005)
Manganese (Mn)	40	FAO/WHO standard (Codex Alimentarius Commission, 1984)
Nickel (Ni)	1.5	Indian standard (Awashthi, 2000)
	1.0	Ministry of Health of China (2005)
Zinc (Zn)	50	Indian standard (Awashthi, 2000)
	60	FAO/WHO standard (Codex Alimentarius Commission, 1984)

Table 9.4: Safe limit of different parameters for agricultural produce (vegetables)

<b>Metals</b>	<b>Safe limit (mg/kg)</b>	<b>References</b>
Cadmium (Cd)	0.3	FAO/WHO standard (Codex Alimentarius Commission 1984)
	1.5	Indian standard (Awashthi 2000)
	0.2	China, SEPA (2005)
Chromium (Cr)	5.0	FAO/WHO standard (Codex Alimentarius Commission 1984)
	20	Indian standard (Awashthi 2000)
	0.5	China, SEPA (2005)
Copper (Cu)	40	FAO/WHO standard (Codex Alimentarius Commission 1984)
	30	Indian standard (Awashthi 2000)
	20	China, SEPA (2005)
Iron (Fe)	450	FAO/WHO standard (Codex Alimentarius Commission 1984)
Lead (Pb)	5.0	FAO/WHO standard (Codex Alimentarius Commission 1984)
	2.5	Indian standard (Awashthi 2000)
	9.0	China, SEPA (2005)
Manganese (Mn)	0.49	IPCS, 1981
Nickel (Ni)	20	FAO/WHO standard (Codex Alimentarius Commission 1984)
	1.5	Indian standard (Awashthi 2000)
	10	China, SEPA (2005)
Zinc (Zn)	60	FAO/WHO standard (Codex Alimentarius Commission 1984)
	50	Indian standard (Awashthi 2000)
	100	China, SEPA (2005)

Table 9.6: Oral reference dose of different metals for an adult human

Metals	ORD (mg/kg bw/day)	References	
Cadmium (Cd)	0.0005	Baars et al., 2001	
	0.001	USEPA (1985)	
Chromium (Cr)	1.50	USEPA (1997 & 2002)	
Cr+6	0.005	Mackenzie et al. (1958)	
Cr+3	2.5	Mackenzie et al. (1958)	
Cobalt (Co)	1.60	FSA, 2003	
Iron (Fe)	0.25	USEPA (1997 & 2002)	
Lead (Pb)	0.004		
Manganese (Mn)	0.14		
Nickel (Ni)	0.02		
Zinc (Zn)	0.30		
Arsenic (As)	0.0003		USEPA (1998c)
Mercury (Hg)			
	Organic	0.0001	Baars et al., 2001, U.S.EPA, 1987
	Inorganic	0.002	Baars et al., 2001
	0.0003	U.S.EPA, 1987	

Table 9.5: Recommended limit of different metals in plant

Metals	Safe limit (mg/kg)	References
Arsenic (As)	0.02-7 (Normal), 5-20 (Critical)	Radojevic and Bashkin (2006)
Cadmium (Cd)	0.1-2.4 (N), 5-30 (C)	
Chromium (Cr)	0.04-14 (N), 5-30 (C)	
Copper (Cu)	5-20 (N), 20-100 (C)	
Iron (Fe)	40-500 (N)	
Lead (Pb)	0.2-20 (N), 30-300 (C)	
Zinc (Zn)	1-400 (N), 100-400 (C)	
Mercury (Hg)	0.005-0.17 (N), 1-3 (C)	
Molybdenum (Mo)	0.03-5 (N), 10-50 (C)	
Nickel (Ni)	0.02-5 (N), 10-100 (C)	
Manganese (Mn))	300-500 (C)	Kabata-Pendias and Pendias, 1992

## BIBLIOGRAPHY

---

- Abdul-Baki, A. and Anderson, J.D. (1973). Vigour determination in Soybean seed by multiple criteria. *Crop Sci.* 13: 630-633.
- Acharya, G.D., Hathi, M.V., Asha, D., Patel and Parmar, K.C. (2008). Chemical properties of groundwater in Bhiloda Taluka Region, North Gujarat, India. *E-Journal of Chemistry*, 5(4): 792-796.
- Akponikpè, P.B.I., Wima, K., Yacouba, H. and Mermoud, A. (2011). Reuse of domestic wastewater treated in macrophyte ponds to irrigate tomato and eggplant in semi-arid West-Africa: Benefits and risks, *Agricultural Water Management*. 98: 834–840.
- Alloway. B.J. (1990). Heavy metals in soils. Blackie Glasgow and London, John Wiley & Sons. Inc. New York.
- Al-Omran. A.M., El-Maghraby, S.E., Nadeem, M.E.A., El-Eter, A.M. and Al-Mohani. (2012). Long term effect of irrigation with the treated sewage effluent on some soil properties of Al-Hassa Governorate, Saudi Arabia. *Journal of Saudi Society of Agricultural Sciences*, 11: 15-18.
- Anon (1985). International rules for seed testing. *Seed Sci. & Technol.*, 13(2): 307-520.
- Aquarec (2006). Integrated concepts for reuse of upgraded wastewater. EVK1-CT-2002-00130 EU funded project. Work Package 2: *Guideline for quality standards for water reuse in Europe*. Salgot, M; Huertas, E. (editors). University of Barcelona.
- Arlosoroff, S. (2002). “Integrated Approach for Efficient Water Use; Case Study; Israel.” *In The World Food Prize International Symposium Held in Des Moines, Iowa*.
- Arnon, D. I. and Stout, P.R. (1939). The essentiality of certain elements in minute quantity for plants with special reference to copper. *Plant Physiol.*, 14: 371-375.

- Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B. and Mittal, N. (2008). Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chemistry*, 111: 811-815.
- Asano, T., Burton, F.L., Leverenz, H.L., Tsuchihashi, R. and Tchobanoglous, G. (2007). Water reuse. Issues, technologies, and applications. Metcalf & Eddy / AECOM. McGraw-Hill, New York.
- Awashthi, S.K. (2000). Prevention of Food Adulteration Act No. 37 of 1954, Central and State Rules as Amended for 1999, Ashoka Law House, New Delhi.
- Baars, A.J., Theelen, R.M.C., Janssen, P.J.C.M., Hesse, J.M., Van-Apeldoorn, M.E., Meijerink, M.C.M., Verdam, L. and Zeilmaker, M.J. (2001). Re-evaluation of human toxicological maximum permissible risk levels. RIVM report 711701 025.
- Bahemuka, T.E. and Mubofu, E.B. (1999). Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dares Salaam, Tanzania. *Food Chemistry*, 66: 63-66.
- Banania, E., Alikhasi, M. and Kouchakzadeh, M. (2010). The Effect of Treated Municipal Wastewater Irrigation in Non-Agricultural Soil on Cotton Plant.
- Bansal, R.L., Nayyar, V.K., Takkar, P.N. (1992). Accumulation and bio-availability of Zn, Cu, Mn and Fe in soils polluted with industrial wastewater. *J. Indian Soc. Soil Sci*, 40: 796-799.
- Barman, S.C., Sahu, R.K., Bhargava, S.K. and Chatterjee, C. (2000). Distribution of heavy metals in wheat, mustard, and weed grown in field irrigated with industrial effluents. *Bulletin of Environmental Contamination and Toxicology*, 64: 489-496.
- Bellack, E. and Schouboe, P.J. (1968). Rapid photometric determination of fluoride with SPADNS-zirconium lake. *Anal. Chem.*, 30: 2032.
- Bennett, W.F. (1993). Nutrient Deficiencies and Toxicities in Crop Plants. St. Paul, MN. APS Press. 202 pages.

- Bergmann, W. (1992). *Nutritional Disorders of Plants*. Gustav Fischer Verlag Jena, Stuttgart/New York.
- Bhardwaj, A.K., Goldstein, D., Azenkot, A. and Levy, G.J. (2007). Irrigation with treated wastewater under two different irrigation methods: Effects on hydraulic conductivity of a clay soil. *Geoderma*, 140: 199-206.
- Bielorai, H., Dasberg, S., Erner, Y. and Brum, M. (1984). The effect of fertigation and partial wetting of the root-zone on production of Shamouti organs. *Proc. Inst. Soc. Citric*. 1: 118-121.
- Bo, S., Mei, L., Tongbin, C., Yuanming, Z., Yunfeng, X., Xiaoyan, L. and Ding, G. (2009). Assessing the health risk of heavy metals in vegetables to the general population in Beijing, China. *Journal of Environmental Sciences*, 21: 1702-1709.
- Bouwer, H. and Chaney, R.L. (1974). Land treatment of wastewater. In: Brady NC, ed. *Advances in agronomy*, New York, Academic Press, 26: 133-176.
- Buat-Menard, P. and Chesselet, R. (1979). Variable influence of the atmospheric flux on the trace metal chemistry of oceanic suspended matter. *Earth Planet Sci Lett*, 42: 398-411.
- Cabrera, F., Clemente, L., Diaz, B. E., Lopez, R. and Murillo, J.M. (1999). Heavy metal pollution of soils affected by the Guadiamar toxic flood. *Sci Total Environ*, 242: 117-29.
- California State Water Resources Control Board (2003). Recycled water use in California. Sacramento, CA, California State Water Resources Control Board, Office of Water Recycling. ([http://www.swrcb.ca.gov/recycling/docs/wrreclaim\\_1\\_atlb.pdf](http://www.swrcb.ca.gov/recycling/docs/wrreclaim_1_atlb.pdf)).
- Cao, H., Chen, J., Zhang, J., Zhang, H., Qiao, L. and Men, Y. (2010). Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China. *Journal of Environmental Sciences*, 22(11): 1792-1799.

- Cao, Z. H. and Hu, Z. Y. (2000). Copper contamination in paddy soils irrigated with wastewater. *Chemosphere*, 41: (1-2, 3-6, 4 pages).
- Carr, R. and Strauss, M. (2001). 'Excreta-related infections and the role of sanitation in the control of transmission', in L. Fewtrell and J. Bartram (eds) *Water Quality: Guidelines, Standards and Health; Assessment of Risk and Risk Management for Water- Related Infectious Disease*, International Water Association (IWA) on behalf on the World Health Organization, London, 89-113 pages.
- Chen, Z.M., Chen, B., Zhou, J.B., Li, Z., Zhou, Y., Xi, X.R., Lin, C. and Chen, G.Q. (2008). A vertical subsurface-flow constructed wetland in Beijing. *Communications in Nonlinear Science and Numerical Simulation*, 13: 1986-1997.
- Chesnin, L. and Yien, C.H. (1950). Turbidimetric determination of available sulphur. *Soil Sci. Soc. Am. Proc.*, 15: 149-51.
- China SEPA (2005). *The limits of Pollutants in Food*. China: State Environmental Protection Administration, GB2762-2005.
- Chung, B.Y., Song, C.H., Park, B.J. and Cho, J.Y. (2011). Heavy metals in Brown Rice (*Oryza sativa* L.) and soil after long-term irrigation of wastewater discharged from domestic sewage treatment plants. *Pedosphere*, 21: 621-627.
- Clesceri, L.S., Greenberg, A.E. and Eaton, A.D. (1998). Standard methods for the examination of water and wastewater. 20<sup>th</sup> ed. American Public Health Association, Washington; ISBN 0875532357, 1325 pages.
- Clesceri, L.S., Greenberg, A.E. and Trussell, R.R. (1996). Standard methods for the examination of water and waste water. *APHA, AWWA and WPCF*, Washington, D.C.
- Codex Alimentarius Commission (1984). Contaminants, Joint FAO/WHO Food Standards Program, Codex Alimentarius, Vol. XVII (1st ed.).
- Coppola, A., Santini, A., Botti, P., Vacc, S., Comegna, V. and Severino, G. (2004). Methodological approach for evaluating the response of soil hydrological

behaviour to irrigation with treated municipal wastewater. *Journal of Hydrology*, 292: 114-134.

Coronado, A.M., Orenes, F.G., Solera, J.M., Arcenegui, V. and Beneyto, J.M. (2011). Short-term effects of treated wastewater irrigation on Mediterranean calcareous soil. *Soil & Tillage Research*, 112: 18-26.

CPCB website [http://cpcb.nic.in/Water\\_Quality\\_Criteria.php](http://cpcb.nic.in/Water_Quality_Criteria.php).

Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z., Qui, Y, et al. (2004). Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environ International*, 30(6): 785-91.

Degens, B.P., Schipper, L.A., Claydon, J.J., Russell, J.M. and Yeates, G.W. (2000). Irrigation of an allophanic soil dairy factory effluent for 22 years: responses of nutrient storage and soil biota. *Australian Journal of Soil Research*, 38: 25-35.

Dere, C., Lamy, I., Oort, F., Baize, D. and Cornu, S. (2006). Trace metal inputs reconstitution and migration assessment in a sandy Luvisol after 100 years of massive irrigation with raw wastewaters. *Comptes Rendus Geoscience*, 338: 565-573.

Eaton, F.M., (1944). Deficiency, toxicity and accumulation of boron in plants. *Agric. Res*, 69: 237-277.

Ensink, J., Simmons, J. and Vander Hoek, W. (2004). Wastewater use in Pakistan: the cases of Haroonabad and Faisalabad. Wallingford, CAB International in association with the International Water Management Institute and International Development Research Centre, 91-102 pages.

European Union (2002). Heavy Metals in Wastes, European Commission on Environment. <http://ec.europa.eu/environment/waste/studies/pdf/heavymetalsreport.pdf>.

FAO (1985). Water quality for agriculture. *Irrigation and Drainage Paper*, 29 Rev. 1. FAO, Rome, 174 pages.

FAO (2002). Crops and drops: making the best use of water for agriculture. Food and Agriculture Organisation of the United Nations, Rome.

- FAO (2010). The wealth of waste -The economics of wastewater use in agriculture, United Nation. *FAO world water report*, 35: 142 pages.
- FAO Wastewater Database (2009). [http://www.fao.org/nr/water/infores\\_databases\\_wastewater.html](http://www.fao.org/nr/water/infores_databases_wastewater.html).
- FAO WATER. (2008). “Water at a Glance: The relationship between water, agriculture, food security and poverty”, Water Development and Management Unit, Food and Agriculture Organization of the United Nations. (<http://www.fao.org/nr/water/docs/waterataglance.pdf>).
- Feigin, A.; Vaisman, I.; Bielorai, H. (1984). Drip irrigation of cotton with treated municipal effluents: II. Nutrient availability in soil. *Journal of Environmental Quality*, 13: 234-238.
- Feizi, M. (2001). Effect of Treated Wastewater on Accumulation of Heavy Metals in Plants and Soil, *ICID-International Workshop on Wastewater Reuse Management*, Seoul, Rep. Korea.
- Food and Agricultural Organization (FAO) of the United Nations. (2008). *AQUASTAT, FAO's Information System on Water and Agriculture*. (<http://www.fao.org/nr/water/aquastat/globalmaps/index.stm> (accessed 2 December 2008)).
- Frolov, I., Alekseev, G. and Danilov, A. (2004). Climate change in polar areas. Proc. World Climate Change Conference, Moscow, Y. Izrael, G. Gruza, S. Semenov and I. Nazarov, Eds., Institute of Global Climate and Ecology, Moscow, 484-490 pages.
- FSA (2003). Safe Upper Levels for Vitamins and Minerals, Expert Group on Vitamins and Minerals, Food Standards Agency, UK. (<http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf>)
- Future Harvest (2001). Wastewater irrigation: economic necessity or threat to health and environment? Consultative Group on International Agriculture Research. (<http://www.futureharvest.org/earth/wastewater.shtml>)

- Galal-Gorchev, H. (1991). Dietary intake of pesticide residues, cadmium, mercury and lead. *Food Additives and Contaminants*, 8: 793-806.
- Gharsallaoui, M., Benincasa, C., Ayadi, M., Perri, E., Khlif, M. and Gabsi, S. (2011). Study on the impact of wastewater irrigation on the quality of oils obtained from olives harvested by hand and from the ground and extracted at different times after the harvesting. *Scientia Horticulturae*, 128: 23-29.
- Ghosh, A.K., Bhatt, M.A. and Agrawal. H.P. (2012). Effect of long-term application of treated sewage water on heavy metal accumulation in vegetables grown in Northern India. *Environ Monit Assess*, 184: 1025-1036.
- Girovich, M.J. (1996). Bio-solids treatment and management: processes for beneficial use. New York, Marcel Dekker, Inc. (Environmental Science and Pollution Control 18).
- Gleick, P.H. (2000). The world's water 2000-2001: the biennial report on freshwater resources. Washington, DC, Island Press.
- Guerra, F., Trevizam, A.R., Muraoka, T., Marcante, N.C. and Brazaca, S.G.C (2012). Heavy metals in vegetables and potential risk for human health. *Sci. Agric*, 69(1): 54-60.
- Gupta, N., Khan, D.K. and Santra, S.C. (2009). Prevalence of intestinal helminth eggs on vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. *Food Control*, 20: 942-945.
- Hakanson, J., Speir, T.W. and Van S.A.P. (1980). An ecological risk index for aquatic pollution control- A sedimentological approach. *Water Res*, 14: 975-1001.
- Halliwell, D.J., Barlow, K.M. and Nash, D.M. (2001). A review of the effects of wastewater sodium on soil physical properties and their implications for irrigation systems. *Australlan Journal of Soil Research*, 39: 1259-1267.
- Harmanescu, M., Alda, L.M., Bordean, D.M., Gogoasa, I. and Gergen, I. (2011). Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. *Chemistry Central Journal*, 5: 64.

- Heidarpour, M., Fard, B.M., Koupai, J.A. and Malekian, R. (2007). The effects of treated wastewater on soil chemical properties using subsurface and surface irrigation methods. *Agricultural Water Management*, 90: 87-94.
- Hough, R.L., Young, S.D., and Crout, N.M.J. (2003). Modelling of Cd, Cu, Ni, Pb and Zn uptake, by winter wheat and forage maize, from a sewage disposal farm. *Soil Use and Management*, 19(1): 19-27.
- Huang, S., Liao, Q., Hua, M., Wu, X., Bi, K., Yan, C., Chen, B. and Zhang, X. (2007). Survey of heavy metal pollution and assessment of agricultural soils in Yangzhong district, Jiangsu Province, China. *Chemosphere*, 67: 2148-2155.
- Hulugalle, N.R., Weaver, T.B., Ghadiri, H. and Hicks, A. (2004). Effect of irrigating cotton with treated sewage effluent on soil properties and deep drainage in a Vertisol. 13th International Soil Conservation Organisation Conference, Brisbane.
- Hussain, A., Murtaza, G., Ghafoor, A., Basra, S.M.A., Qadir, M. and Sabir, M. (2010). Cadmium Contamination of Soils and Crops by Long Term Use of Raw Effluent, Ground and Canal Waters in Agricultural Lands. *International Journal of Agriculture & Biology*, 12(6): 851-856.
- Idelovitch, E. and Michael, M. (1984). A new approach to an old method of wastewater reuses. *J. Water Pollut. Control Fed.*, 56: 9300 pages.
- Ikeda, M., Zhang, Z.W., Shimbo, S., Watanabe, T., Nakatsuka, H., Moon, C.S., Matsuda- Inoguchi, N., Higashikawa, K., (2000). 'Urban population exposure to lead and cadmium in east and south-east Asia'. *Science of the Total Environment*, 249: 373-384.
- Ingwersen, J. and Streck, T. (2006). Modelling the environmental fate of cadmium in a large wastewater irrigation area. *Journal of Environmental Quality*, 35:1702-1714.
- IPCS (1981). World Health Organization, International Programme on Chemical Safety. Manganese, Geneva, (Environmental Health Criteria 17).

- Iyengar, V. and Nair, P., (2000). 'Global outlook on nutrition and the environment: meeting the challenges of the next millennium'. *Science of the Total Environment*, 249: 331-346.
- Jackson, M.L. (1973). *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi.
- Jalali, M., Merikhpour, H., Kaledhonkar, M.J. and Van Der Zee, S.E.A.T.M. (2008). Effects of wastewater irrigation on soil sodicity and nutrient leaching in calcareous soils. *Agricultural Water Management*, 95: 143-153.
- Jansson, J. and I. Oborn, (2000). Cadmium content of swedish carrots and the influence of soil factors. *Acta Agric. Scandinavica*, 50: 49-56.
- Jiménez, B. and Asano, T. (2008). 'Water reclamation and reuse around the world', *Water Reuse: An International Survey of Current Practice, Issues and Needs*, IWA Publishing, London, 648 pages.
- Jopony, M., and Young, S.D. (1994). The Solid $\rightleftharpoons$ Solution Equilibria of Lead and Cadmium in Polluted Soils. *European Journal of Soil Science*, 45(1): 59-70.
- Jouzdan, O., Fares, F. and Abedalgawad, G. (2007). The Effect of using Urban Treated and Untreated Effluents on Soil and Agricultural Crops Pollution in Syria (Damascus-Ghouta). *American-Eurasian J. Agric. & Environ. Sci.*, 2(1): 51-61.
- Kabata-Pendias, A., Pendias, H. (1992). *Trace elements in soils and plants*. 2nd ed. CRC Press, Inc. Boca Raton, Florida, 365 pages.
- Kadlec, R.H. and Knight, R.L. (1996). *Constructed wetlands*. Boca Raton, F L: Lewis Publishers - CRC Press, 298 pages.
- Kalavrouziotis, I.K., Robolas, P., Koukoulakis, P.H. and Papadopoulos, A.H. (2008). Effects of municipal reclaimed wastewater on the macro and micro-elements status of soil and of *Brassica oleracea* var. *Italica*, and *B. oleracea* var. *Gemmifera*, *Agricultural Water Management*, 95: 419-426.

- Kashyap, A. (2004). Water governance: learning by developing adaptive capacity to incorporate climate variability and change. *Water Science and Technology*, 19(7): 141-146.
- Katerji, N., Van-Hoorn, J.W., Hamdy, A. and Mastrorilli, M. (2003). Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. *Agric. Water Manage*, 62(1): 37-66.
- Kaur., R., Dheer, G., Laishram, G., Ningthoujam, D. and Kumar, P. (2012). Nutrient and trace metal removal efficiency of small scale (batch fed) vertical flow municipal wastewater treatment wetlands (REF NO: ECOS2012\_0163). Accepted for Oral Presentation at 4<sup>th</sup> International Eco-Summit on Ecological Sustainability-Restoring the Planets Ecosystem Services.
- Kent, N.L. and Everes, A.D. (1990). *Technology of Cereals: An Introduction for Students of Food Science and Agriculture*. 4th ed., Pergamon press, New York.
- Khan, M.J., Jan, M.T., Farhatullah, Khan, F., N.U., Arif, M., Perveen, S., Alam, S. and Jan, A.U. (2011). The Effect of Using Waste Water For Tomato. *Pak. J. Bot*, 43(2): 1033-1044.
- Khillare, P.S., Jyethi, D.S. and Sarkar, S. (2012). Health risk assessment of polycyclic aromatic hydrocarbons and heavy metals via dietary intake of vegetables grown in the vicinity of thermal power plants. *Food and Chemical Toxicology*, 50: 1642-1652.
- Khodapanah, L., Sulaiman, W.N.A. and Khodapanah, D. N. (2009) "Groundwater Quality Assessment for Different Purposes in Eshtehard District, Tehran, Iran." *European Journal of Scientific Research*, 36 (4): 543-553.
- Kiziloglu, F.M., Turan, M., Sahin, U., Kuslu, Y. and Dursun, A. (2008). Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (*Brassica oleracea* L.var. botrytis) and red cabbage (*Brassica oleracea* L. var. rubra) grown on calcareous soil in Turkey. *Agricultural water management*, 95: 716-724.

- Krijger, G. C., Vliet, P. M., & Wolterbeek, H. T. (1999). Metal speciation in xylem exudate of *Lycopersicon esculantum*. *Plant and Soil*, 212: 165-173.
- Lal, R., Kimble, J.M., Follett, R.F., Cole, C.V. (1998). The potential of US cropland to sequester carbon and Mitigate the Greenhouse effect. Ann Arbor Press, Chelsea, MI.
- Lee, S. R. and Lee, M. G. (2001). Contamination and risk analysis of heavy metals in Korean foods. *J. Food Hyg. Safety*. (in Korean). 16: 324-332.
- Lindsay, W. L. and Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Amer.J*, 42: 421-428.
- Liu, C.Z. (2002). *Suggestion on Water Resources in China Corresponding with Global Climate Change*. *China Water Resources*, 2: 36-37.
- Liu, J., Zhang, X.H., Tran, H., Wang, D.Q. and Zhu, Y.N. (2011). Heavy metal contamination and risk assessment in water, paddy soil, and rice around an electroplating plant. *Environ Sci Pollut Res*, 18: 1623-1632.
- Liu, W., Zhao, J., Ouyang, Z., Soderlund, L. and Liu, G. (2005). Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environment International*, 31: 805-812.
- Liu, J., Dorjderem, A., Fu, J., Lei, X., Liu, H., Macer, D., Qiao, Q., Sun, A., Tachiyama, K., Yu, L. and Zheng, Y. (2011). *Water Ethics and Water Resource Management*, UNESCO, Bangkok, 84 pages.
- Luederitz, V., Eckert, E., Lange-Weber, M., Lange, A., and Gersberg, R. (2001). Nutrient removal efficiency and resource economics of vertical flow and horizontal flow constructed wetlands. *Ecological Engineering*, 18: 157-171.
- Lzarova, V. and Bahri, A. (2005). *Water Reuse for Irrigation: Agriculture, Landscape and Turf Grass*, CRC Press, Boca Raton, FL.
- Mackenzie, R.D., Byerrum, R.U. and Decker, C.F. (1958). Chronic toxicity studies. II. Hexavalent and trivalent chromium administered in drinking water to rats. *Am. Med. Assoc. Arch Ind. Health*, 18: 232-234.

- Macnicol, R.D. and P.H.T. Beckett, (1985). Critical tissue concentrations of potentially toxic elements. *Plant Soil*, 85: 107-129.
- Magadza, C. (2000). Climate change impacts and human settlements in Africa: prospects for adaptation. *Environmental Monitoring and Assessment*, 61(1), 193-205.
- Magdaleno, H.F., Villa, O.R.M., Saenz, E.M., Bolaños, M.D.C.O. and Olivas, A.L.B. (2011). Heavy metals in agricultural soils and Irrigation wastewater of Mixquiahuala, Hidalgo, Mexico. *African Journal of Agricultural Research*, 6(24): 5505-5511.
- Magesan, G.N., Williamson, J.C., Yeates, G.W. and Lloyd-Jones, A.Rh. (2000). Wastewater C/N ratio effects on soil hydraulic conductivity and potential mechanisms for recovery. *Bioreso. Technol*, 71, 21-27.
- Magesan, G.N., Williamson, J.C., Yeates, G.W., Sparling, G.P., Schipper, I.A. and Lloyd- Jones, A.Rh. (1999). Hydraulic conductivity in soils irrigated with wastewaters of differing strengths: field and laboratory studies. *Aust. J. Soil Res.* 37: 391-402.
- Mandal, U.K., Bhardwaj, A.K., Warrington, D.N., Goldstein, D., Tal, A.B. and Levy, G.J. (2008b). Changes in soil hydraulic conductivity, runoff, and soil loss due to irrigation with different types of saline–sodic water. *Geoderma*, 144: 509-516.
- Mandal, U.K., Warrington, D.N., Bhardwaj, A.K., Bar-Tal, A., Kautsky, L., Minz, D., Levy, G.J. (2008a). Evaluating impact of irrigation water quality on a calcareous clay soil using principal component analysis. *Geoderma*, 144: 189-197.
- Masona, C., Mapfai, L., Mapurazi, S. and Makanda, R. (2011). Assessment of Heavy Metal Accumulation in Wastewater Irrigated Soil and Uptake by Maize Plants (*Zea Mays* L) at Firlé Farm in Harare. *Journal of Sustainable Development*, 4(6): 132-137.
- McKone, T.E. and Ryan, P.B. (1989). Human exposure to chemicals through food chains: Uncertainty analysis. *Environ. Sci. Technol.*, 23: 1154-1163.

- McLaughlin, M.J., K.G. Tiller and M.K. Smart, (1997). Speciation of cadmium in soil solutions of saline/sodic soils and relationship with cadmium concentrations in potato tubers. *Australian J. Soil Res.*, 35: 1-16.
- Mikkelsen, R. and Camberato, J. (1995). Potassium, sulphur, lime and micronutrient fertilizers. In: Rechcigl J, ed. Soil amendments and environmental quality. Chelsea, MI, Lewis Publishers.
- Minhas, P.S., Sharma, N., Yadav, R.K. and Joshi, P.K. (2006). Prevalence and control of pathogenic contamination in some sewage irrigated vegetable, forage and cereal grain crops. *Bioresource Technology*, 97: 1174-1178.
- Ministry of Health of the People's Republic of China (MHC) (2005). Maximum levels of contaminants in foods, GB 2762-2005.
- Mishra, A. and Tripathi, B.D. (2008). Heavy metal contamination of soil, and bioaccumulation in vegetables irrigated with treated waste water in the tropical city of Varanasi, India. *Toxicological & Environmental Chemistry*, 90(5): 861-871.
- Mojiri, A. (2011). Effects of Municipal Wastewater on Physical and Chemical Properties of Saline Soil. *J. Biol. Environ. Sci.*, 5(14): 71-76.
- Mukherjee, A., and Mishra, V.K. (2008). Bioaccumulation of heavy metal in crops irrigated with secondary treated sewage wastewater in surrounding villages of Varanasi city. *Res. Environ. Life Sci*, 1(3): 103-108.
- Murtaza, G., Ghafoor, A. and Qadir, M. (2008). Accumulation and implications of cadmium, cobalt and manganese in soils and vegetables irrigated with city effluent. *J. Sci. Food Agric.*, 88(1): 100-107.
- National Academy of Sciences and National Academy of Engineering (1972). Water quality criteria, US Environmental Protection Agency, Washington DC, Report No. EPA-R: 373-033, 592 pages.
- National Research Council (1996). Use of reclaimed water and sludge in food crop production. Washington, DC, National Academy Press, 64-65 pages.

- National Research Council (1998). Issues in potable reuse: the viability of augmenting drinking water supplies with reclaimed water. Washington, DC, National Academy Press.
- Neilsen, G.H., Stevenson, D.S., and Fitzpatrick, J.J. (1989a). The effect of municipal wastewater irrigation and rate of N fertilization on petiole composition, yield and quantity of Okanagan Riesling grapes. *Can.J.Plant Sci.*, 69: 1285-1294.
- Neilsen, G.H., Stevenson, D.S., Fitzpatrick, J.J. and Brownlee, C.H. (1989c) Nutrition and yield of young apple trees irrigated with municipal wastewater. *J.Amer.Soc.Hort.Sci.*, 114: 377-383.
- Neilsen, G.H., Stevenson, D.S., Fitzpatrick, J.J. and Brownlee, C.H. (1991). Soil and sweet cherry responses to irrigation with wastewater. *Can. J. Sci. Soil Soc.*, 71: 31-41.
- Neilsen, G.H., Stevenson, D.S., Fitzpatrick, J.J. and C.H. Brownlee (1989b) Yield and plant nutrient content of vegetables trickle-irrigated with municipal wastewater. *HortScience*, 24: 249-252.
- Nielsen, G. H., Hogue, E. J., Nielson, D., and Zebarth, B. J. (1998). Evaluation of organic wastes as soil amendments for cultivation of carrot and chard on irrigated sandy soils. *Canadian Journal of Soil Science*, 78: 217- 225.
- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *U.S. Dep. of Agric. Circ.*, 939 pages.
- Pachauri, R. (2004). Climate change and its implications for development: the role of IPCC assessments. *Institute of Development Studies Bulletin*, 35: 11.
- Palaniswami, C. and Sree Ramulu, U.S. (1994). Effect of continuous flow of effluent in a channel on the available nutrient status and on certain soil enzyme activities of the adjoining fields. *J. Indian Soc. Soil Sci*, 42: 42-468.
- Parvan, M., Danesh, S. and Alizadeh, A. (2009). Effects of irrigation with treated wastewater on Chemical Soil Properties. *Geophysical Research Abstracts*, EGU (2): 2009-2981.

- Patankar, S.N. (2001). Reuse of treated wastewater and sludge for Agriculture in India – case study. ICID International Workshop on Wastewater Reuse Management.
- Pescod, M. (1992). Wastewater treatment and use in agriculture. Bull FAO (Rome) 47: 125 pages.
- Pierzynski, G.M., Sims, J.T. and Vance, G.F. (2000). Soils and Environmental Quality, second ed. CRC Press, LLC, NW Corporate Blvd., Boca Raton, FL.
- Pratt, P.F. (1972). Quality criteria for trace elements in irrigation waters. California Agricultural Experiment Station, 46 pages.
- Puschenreiter, M., Horak, O., Friesl, W., and Hartl, W. (2005). Low-cost agricultural measures to reduce heavy metal transfer into the food chain—A review. *Plant Soil Environment*, 51: 1-11.
- Qadir, M., Sharma, B.R., Bruggeman, A., Allah, R.C. and Karajeh, F. (2007). Non-conventional water resources and opportunities for water augmentation to achieve food security in water scarce countries. *Agricultural Water Management*, 87: 2-22.
- Radojevic M. and Bashkin V.N. (2006). Practical Environmental Analysis. Royal Society of Chemistry, Cambridge, 389 pages.
- Rahman, M. A., Hasegawa, H., Rahman, M. M., Islam, M. N., Majid, M. M. A. and Tasmen, A. (2007). Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere*, 67: 1072-1079.
- Rattan, R.K., Datta S.P., Chhonkar, P.K., Suribabu, K. and Singh, A.K. (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. *Agriculture, Ecosystems and Environment*, 109: 310-322.
- Rattan, R.K., Datta, S.P., Chandra, S. and Saharan, N. (2002). Heavy metals and environmental quality: Indian scenario. *Fertil. News*, 47(11): 21-40.

- Rebora, C., Lelio, H., Gómez, L. and Ibarguren, L. (2010). Waste-Water Use in Energy Crops Production, Waste Water - Treatment and Reutilization, Argentina.
- Reed, S.C. and Brown, D. (1995). Subsurface flow wetlands - A performance evaluation. *Water Environmental Research*, **67** (2): 244-248.
- Richards, L.A. (1954). Diagnosis and improvement of saline and alkali soil, U.S. Department of Agriculture. *Agriculture hand book*, Washington DC, 60 pages.
- Saha, J.K., Panwar, N., Srivastava, A., Biswas, A.K., Kundu, S. and Rao, S.A. (2010). Chemical, biochemical and biological impact of untreated domestic sewage water use on Vertisol and its consequences on wheat (*Triticum aestivum*) productivity. *Environ Monit Assess*, 161: 403-412.
- Scott, C. A, Faruqui, N. I. and Raschid-Sally, L. (2004). 'Wastewater use in irrigated agriculture: Management challenges in developing countries', *Wastewater Use in Irrigated Agriculture: Confronting the Livelihood and Environmental Realities*, CABI Publishing, Wallingford, UK, 1-10 pages.
- Scott, C., Drechsel, P., Raschid-Sally, L., Bahri, A., Mara, D., Redwood, M., and Jiménez, B. (2010). "Wastewater irrigation and health: challenges and outlook for mitigating risks in low-income countries." In *Wastewater Irrigation and Health: Assessing and Mitigating Risk in Low-Income Countries*, London. 381-94 pages.
- Sharma, R. K., Agrawal, M. and Marshall, F. (2007). Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotox. Environ. Safe*, 66: 258-266.
- Shende, G.B. (1985). Status of wastewater treatment and agricultural reuse with special reference to Indian experience and research and development needs. In: Pescod MB, Arar A, ed., *Proceeding of the FAO Regional seminar on the Treatment and use of sewage effluent for irrigation*, Nicosia, 7-9 Oct. 1985, London, Butterworths, 185-209 pages.

- Si, W., Ji, W., Yang, F., Lv, Y., Wang, Y. and Zhang, Y. (2011). The function of constructed wetland in reducing the risk of heavy metals on human health, *Environ Monit Assess.* 181: 531-537.
- Singer, M.J. and Eshel, G. (2003). Enhancing Inorganic carbon sequestration by irrigation management in California, *Kearney Foundation of Soil Science: Soil Carbon and California's Terrestrial Ecosystems* Final Report: 2001010, 1/1/2002-12/31/2003. (<http://kearney.ucdavis.edu/OLD%20MISSION/2001%20final%20reports-research%20projects/2001010SingerFINALkms.pdf>).
- Singh, A., Sharma, R.K., Agrawal, M. and Marshall, F.M. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology*, 48: 611-619.
- Singh, K.P., Mohon, D., Sinha, S. and Dalwani, R., (2004). Impact assessment of treated/untreated wastewater toxicants discharge by sewage treatment plants on health, agricultural, and environmental quality in wastewater disposal area. *Chemos*, 55: 227-255.
- Singh, P.K., Deshbhratar, P.B., and Ramteke, D.S. (2012). Effects of sewage wastewater irrigation on soil properties, crop yield and environment, *Agricultural Water Management*, 103: 100-104.
- Singh, S. and Kumar, M. (2006). Heavy metal load of soil, water and vegetables in peri-urban Delhi. *Environmental Monitoring and Assessment*, 120: 71-79.
- Smit, J. and Nasr, J., (1992). Urban agriculture for sustainable cities: using wastes and idle land and water bodies as resources. *Environment and Urbanisation*, 4(2): 141-152.
- Smith, R.G. and Schroeder, E.D. (1985). Field studies of the overland flow process for the treatment of raw and primary treated municipal wastewater. *J. Water Pollut. Control Fed.*, 57: 785 pages.

- Stewart, E.A., Max-Grimshaw, H., Parkinson, J.A. and Quarmby, C. (1974). *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publications, Osney Mead, Oxford, 165 pages.
- Strauss, M. and Blumenthal, U.J. (1990). Human waste use in agriculture and aquaculture: utilization practices and health perspectives. *IRCWD Report 09/90*. International Reference Centre for Waste Disposal (IRCWD), Duebendorf, Germany, 48 pages.
- Surdyk, N., Cary, L., Blagojevic, S., Jovanovic, Z., Stikic, R., Radovic, B.V., Zarkovic, B., Sandei, L., Pettenati, M. and Kloppmann, W. (2010). Impact of irrigation with treated low quality water on the heavy metal contents of a soil-crop system in Serbia. *Agricultural Water Management*, 98: 451-457.
- Sutherland, R.A. (2000). Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. *Environ. Geol.*, 39: 611-27.
- Tarchitzky, J., Golobati, Y., Keren, R. and Chen, Y. (1999). Wastewater effects on mont-morillonite suspensions and hydraulic properties of sandy soil. *Soil Sci. Soc. Am. J.*, 63, 554-560.
- Tinker, P.B. (1981). Levels, distribution and chemical forms of trace elements in food plants. *Philos. Trans. B.*, 294 :41-55.
- Tomlison, L, Wilson, G., Harris, R. and Jeffrey, D.W. (1980). Problems in the assessments of heavy metal levels in estuaries and formation of a pollution index. *Helgol Meeresunters*, 33: 566-75.
- Truu, M., Truu, J. and Heinsoo, K. (2009). Changes in soil microbial community under willow coppice: The effect of irrigation with secondary-treated municipal wastewater. *Ecological Engineering*, 35: 1011-1020.
- Tu'rkdogan, M.K., Fevzi, K., Kazim, K., Ilyas, T. and Ismail, U. (2003). 'Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey'. *Environmental Toxicology and Pharmacology* 13, 175-179.

- Tye, A.M., Young, S.D., Crout, N.M.J., Zhang, H., Preston, S., Barbosa-Jefferson, V.L., Davison, W., McGrath, S.P., Paton, G.I., Kilham, K., and Resende, L. (2003). Predicting the activity of  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  in soil pore water from the radio-labile metal fraction. *Geochimica et Cosmochimica Acta*, 67(3): 375-385.
- U.S. Environmental protection agency (1998c). Iris File, *Arsenic, inorganic*. April 10, 1998.
- U.S. EPA. (1985). Updated Mutagenicity and Carcinogenicity Assessment of Cadmium. Addendum to the Health Assessment Document for Cadmium (EPA 600/B- B1-023). EPA 600/B-83-025F.
- U.S. EPA. (1987). Peer Review Workshop on Mercury Issues. Environmental Criteria and Assessment Office, Cincinnati, OH. Summary report. October 26-27.
- UN WWAP (2003). The World Water Development Report-1: Water for People, Water for Life, UNESCO, Paris, France.
- US Environmental Protection Agency (USEPA) (1997). Exposure factors handbook—general factors. EPA/600/P-95/002Fa, vol. I. Office of Research and Development, National Center for Environmental Assessment, Washington.
- US Environmental Protection Agency (USEPA) (2002). Region 9, preliminary remediation goals. USEPA, Washington.
- US Salinity Laboratory (1954). “Diagnosis and Improvement of Saline and Alkaline Soils,” Department of Agriculture, Handbook No. 60, 160 pages.
- USEPA (1992). Technical support document for land application of sewage sludge. Prepared for Office of Water, United States Environment Protection Agency, by Eastern Research Group, Lexington, MA.
- USEPA (1992a). Guidelines for water reuse. Washington, DC, United States Environmental Protection Agency and United States Agency for International Development (Technical Report No. 81)

- USEPA (2000). Risk based Concentration Table. Philadelphia PA: United States Environmental Protection Agency, Washington DC.
- Van-der-Hoek, W. (2004). A Framework for a Global Assessment of the Extent of Wastewater Irrigation: The Need for a Common Wastewater Typology, CAB International, International Water Management Institute, International Development Research Centre, Trowbridge 11-24 pages.
- Varalakshmi, L.R. and Ganeshamurthy, A.N. (2010). Heavy metal contamination of water bodies, soils and vegetables in peri urban areas of Bangalore city of India. *World Congress of Soil Science, Soil Solutions for a Changing World*.
- Walker, C. and Lin, H.S. (2008). Soil property changes after four decades of wastewater irrigation: A landscape prospective. *Catena*, 73: 63-74.
- Walker, J.M. (1988). Regulation by other Countries in Foods and the Human Environment, *Proc. No. 2 "Cadmium Accumulation in Australian Agriculture" National Symposium, Canberra, 1-2 March*, Australian Government Publication Service, Canberra, 176-185 pages.
- Walkley, A. and Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid titration method. *Soil Sci.*, 37: 29-38.
- Wang D.K.Y., Ewing A.G. (1989). Anodic Stripping Voltammetry at Mercury Films Deposited on Ultrasmall Carbon-Ring Electrodes, *Anal.Chem.* 62: 2697 pages.
- Wang, S., Nan, Z., Liu, X., Li, Y., Qin, S. and Ding, H. (2009). Accumulation and bioavailability of copper and nickel in wheat plants grown in contaminated soils from the oasis, northwest China. *Geoderma*, 152: 290-295.
- WAPDA (1981). Soil salinity Survey, Soil Salinity and Water table Survey Directorate, Planning Division, Lahore, Pakistan, vol. 1.
- WHO (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. Technical Report No. 778. WHO, Geneva, 74 pages.
- WHO (1998). Environmental Management for vector control. World Health Organization, Geneva.

- WHO (2006). Guidelines for the Safe use of wastewater, Excreta and grey water. (Wastewater Use in Agriculture), WHO, Geneva, Switzerland, 2: 222 pages.
- WHO and UNICEF (2000). (World Health Organization/United Nations Children's Fund). *Global Water Supply and Sanitation Assessment Report*, New York.
- Willis, J.B. (1962). Determination of lead and other heavy metals in urine by atomic absorption spectrophotometry. *Anal. Chem.*, 34: 614 pages.
- Yadav, R.K., Goyal, B., Sharma, R.K. Dubey, S.K. and Minhas, P.S. (2002). Post-irrigation impact of domestic sewage effluent composition of soils, crops and groundwater- a case study. *Environment International*, 28: 481-486.
- Yan, Z., Wen, Z.H., Cheng, Z.SU. and Gang, C.Z. (2008). Soil Microbial Characteristics Under Long-Term Heavy Metal Stress: A Case Study in Zhangshi Wastewater Irrigation Area, Shenyang. *Pedosphere*, 18(1): 1-10.
- Yoon, C.G. and Kwun, S.K. (2001). Feasibility Study of Reclaimed Wastewater Irrigation to Paddy Rice Culture in Korea, *ICID-International Workshop on Wastewater Reuse Management*, Seoul, Rep. Korea.
- Zhang, Y.L., Dai, J.L., Wang, R.Q. and Zhang, J. (2008). Effects of long-term sewage irrigation on agricultural soil microbial structural and functional characterizations in Shandong, China. *European Journal of Soil biology*, 44: 84-91.