Mobility and Uptake of Zn, Cd, Ni and Pb in Sludge-amended Soils Planted to Dryland Maize and Irrigated Maize-Oat rotation

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Abbreviations:TMT, total maximum threshold; TIL, total investigative level; MAT, maximum available threshold; EDTA, Ethylenediaminetetraacetic acid; ERWAT, East Rand Water Care Works; WRC, Water Research Commission

ABSTRACT

Sludge application to agricultural lands is often limited, mainly because of concerns about metal accumulation in soils and uptake by crops. The objective of the study was to test the hypotheses that in the short to medium term (5-10 years) the application of good quality sludge according to crop nitrogen requirements: i) will not lead to significant accumulation of water soluble metal fractions in soil, ii) mobility and uptake of metals is higher under irrigated than dryland systems, and iii) metal concentrations in plant tissue could reach phytotoxic levels before the soil reaches environmental threshold levels. Field plots were arranged in a randomized complete block design comprising four replications of three treatments (0, 8, and 16 Mg ha^{-1} yr^{-1} anaerobically digested municipal sludge) planted to dryland maize and irrigated maize-oat rotation. Soil and plant samples were collected following 7 years of treatment application for selected metal analyses. A large fraction of the Zn, Ni, and Pb in the soil profile was EDTA extractable (46 to 79%). Saturated paste extractable fractions of Cd and Pb were <1 mg kg^{-1}. Plant uptake of Cd, Pb and Ni under irrigation was double that for dryland systems. Concentrations of the metals considered in plant tissue of both cropping systems remained well below phytotoxic levels, except for Zn under dryland maize that received 16 Mg sludge ha^{-1} yr^{-1}. Metal concentrations in the soil remained far below total maximum threshold levels. Therefore, hypotheses 1 and 3 were accepted for the metals considered and hypothesis 2 was rejected for Zn.

Introduction

The use of sewage sludge for crop production is a well-known practice around the world because of its soil conditioning effect and as a source of low grade fertilizer (Gasco and Lobo, 2007; Herselman, 2010). Sewage sludge, when applied to agricultural soils, can substitute inorganic fertilizers and improve soil physical properties. However, one of the main concerns for sustainable use of municipal sewage sludge on agricultural soils, is the long term build-up of trace metals (Smith, 2009). Some trace metals (e.g., Cu and Zn) are essential for plant and animal health. However, concentrations exceeding threshold levels have the potential to cause toxicity to plants and animals (Sterritt and Lester, 1984). Other heavy metals (e.g., Cd and Pb) are not known to be essential to plants and animals. Toxicity, however, occurs when metals are concentrated in the environment above threshold levels.

Finding environmentally acceptable, socially responsible, and economically feasible ways of using municipal sewage sludge has received much attention from both the research community and regulatory agencies, as well as from the general public. Land application provides a means of supplying nutrients, such as nitrogen (N) and phosphorus (P), and organic matter (OM), and can be both agriculturally useful and environmentally responsible. However, application of sludge has led to concerns on how potential contaminants (heavy metals, organic contaminants and pathogens) may pollute the groundwater underlying application areas. Generally the natural background concentration of heavy metals in soils plays a determining role in the level of threat to groundwater pollution from any anthropogenic additions such as through the application of sludge. The background concentration of a soil is defined as the normal chemical composition of an earth material prior to its contamination (Korte, 1999). The background concentration is a function of the parent material and soil formation processes and is therefore highly variables across regions. For instance, in many South African soils, the natural background concentrations of Ni, Zn, and Pb is high (Herselman et al., 2005). Therefore, extreme caution is needed when applying sludge to agricultural soils.

Previous studies have shown that heavy metal uptake by crops increases as sludge application rate increases (Hinesly et al., 1978; Bidwell and Dowdy, 1987; Kiemnec et al., 1990; Logan et al., 1997). Heavy metal uptake by crops is influenced by metal concentration in the soil, as well as soil physical and chemical properties (Merrington, et al., 2003). Other studies reported an increase in soil metal concentrations as sludge application rate increased in fields where crops were not planted (Williams et al., 1984). Few studies have investigated the effect of sludge application on crop heavy metal uptake and accumulation in the soil profile (Soon et al.,1980; MacLean et al., 1987; Granato et al., 2004; Fuentes et al., 2006). The movement of heavy metals in sludge amended soils (Emmerich et al., 1982; Yingming and Corey, 1993), mass balance and distribution of sludge-borne trace elements following long-term application of sewage sludge (Baveye et al., 1999), and influence of sewage sludge application on soil properties, distribution and availability of heavy metal fractions (Tsadilas et al., 1995) has also been investigated. However, to our knowledge, there is no information on the effect of soil water availability (dryland vs. intensive irrigated cropping systems) on the dynamics of metals in the soil-plant system (metal crop uptake and partitioning between plant organs, fate and mobility within the soil system) from class A (U.S. EPA, 1995), or class A1a (South Africa, Snyman and Herselman (2006)) sludge amended soils under controlled short to medium term field experiments. In addition, the unwritten rule of using environmental soil contamination threshold values as phytotoxicity indicators needs to be investigated. Similarly, little is known about the dynamics of these metals with respect to the relationship between total concentration, plant available and water soluble fractions of metals from sludge amended soils under dryland vs. irrigated systems. This study focuses on Zn, Cd, Ni and Pb, which are four of the eight elements of concern listed in the South African agricultural sludge guideline (Snyman and Herselman, 2006). These four are selected because of their potential reactivity, toxicity, mobility and availability in South African soils and sludges.

The objective of the study was to test the hypotheses that in the short to medium term (5-10 years) the application of agricultural quality sludge according to crop nitrogen requirements: i) will not result in a significant increase in the water soluble content of heavy metals in the soil, ii) mobility and uptake of heavy metals for an irrigated maize-oat rotation will be higher than for dryland maize, and iii) the concentration of heavy metals in plant tissue could reach phytotoxic levels before the soil reaches environmental threshold levels.

Materials and Methods

Field Site Description

The study was conducted at the East Rand Water Care Works (ERWAT), Johannesburg, Gauteng, South Africa (26º 01’ 01” S; 28º 16’ 55” E; altitude 1577 m above sea level). The area has a long term annual average rainfall of 700 mm, mainly from October to March. The soil of the experimental site is a clay loam (Hutton; Soil Classification Working Group, 1991) having a clay content of 36 to 46%, and pH (H_2O) of 5.3 to 6.1. In the beginning of the study, the cation exchange capacity of the soil (ammonium acetate extract) was 12 $cmol_c$ kg^{-1} and the electrical conductivity of the saturation paste extract ranged from 8 mS m^{-1} at 1.2 m depth, to 36 mS m^{-1} in the top 0.3 m soil layer. Initial concentration of Zn, Cd, Ni, Pb and other chemical properties of the soil for the study site are provided in Table 1.

Table 1 Initial chemical properties and concentration of selected metals in a clay loam Hutton soil.

Zn	**Cd**	**Ni**	**Pb**	**P-Bray1**	**Exchangeable K**	**N**	**Organic matter**
mg kg^{-1}						%	
10.35	0.018	29.48	14.85	43.9	24.63	0.13	2.98

Field Trials and Treatments

Plots of 25 m^2 were arranged in a randomized complete block design comprising four replications of three sludge application rate treatments. The trial was laid out to accommodate widely different levels of bio-solid application to high and low productivity-cropping systems. It consisted of two farming systems namely: dryland maize and an irrigated maize-oat rotation. The two contrasting cropping systems were selected to represent a dryland (rainfed) farming system on the one hand and an intensive irrigated system on the other hand. This created a range of conditions affecting nutrient and metal mobility and uptake. Maize was selected as test crop because it is one of the most widely cultivated crops across the globe and accounts for 51% of the cultivated land in South Africa (FAO, 2005). Oats were planted as a rotation crop because of its dual benefits: healthy grain for human beings (Peterson, 1992) and a high quality fodder for animals (Schrickel et al., 1992) and can be planted (under irrigation) during winter as rotation crop in the summer rainfall areas or can be alternated with legume crops in summer.

The treatments for dryland maize and irrigated maize-oat rotation consisted of two sludge rates (8 and 16 Mg ha^{-1} yr^{-1}), and a zero control. The value of 8 Mg ha^{-1} yr^{-1} represents the annual agricultural upper limit of the 1997 South African sludge guideline (WRC, 1997) which has recently been raised to 10 Mg ha^{-1} yr^{-1} (Snyman and Herselman, 2006). Sludge rates of 16 Mg ha^{-1} yr^{-1} represent double the former norm. Sludge was applied since the 2004/05 until the 2011/12 summer season. For the irrigated maize-oat rotation, the annual sludge application was split into two, with half applied to both crops at planting. For dryland maize, however, the entire amount was applied at the beginning of the season before planting. Sludge was broadcast and immediately incorporated into the top soil (0.3 m) with a manually operated, diesel powered rotovator (Agria). After sludge incorporation, the soil was leveled using rakes and maize (CV. PAN 6966) was planted in 0.9 m rows at rates of 80000 seeds per hectare under irrigation and half this rate under dryland conditions. Each plot consisted of 6 rows 5 m in length, with the outer row on either side taken as border rows. After harvesting irrigated maize, sludge was applied accordingly and again incorporated, after which oats were planted. Oats were planted at a rate of 90 kg ha^{-1} using a hand-drawn planter with double disk openers. Each oat plot consisted of 15 rows, spaced 0.3 m apart and 5 m in length, with two border rows on either side.

Sludge Characteristics

The sludge used in this study was anaerobically digested and paddy-dried. According to the current South African sludge guideline (Snyman and Herselman, 2006), this sludge is classified as pollutant class "A" because of its low heavy metal content (Table 2). Based on the microbiological report from the East Rand Water Care Works (ERWAT) laboratory, the sludge can also be classified as microbiological class “a”. Considering the low odour and vector attraction characteristics of the sludge, it can also be classified as stability class 1.

Table 2 New three-tier system for the classification of South African sludges (Snyman and Herselman, 2006).

Pollutant Class (mg kg^{-1})			
	A	**B**	**C**
Zn	<2800	2800-7500	>7500
Cd	<40	40-85	>85
Ni	<420	420	>420
Pb	<300	300-840	>840
Stability Class			
	1	**2**	**3**
	Low odour and vector attraction	Medium attraction	High odour and vector attraction
Microbiological Class			
	a	**b**	**c**
Faecal coliform (CFU/g_{dry})	$<10^4$	10^6-10^7	$> 10^7$
Helminth ova (Viable ova/g_{dry})	<1	1-4	>4

The current South African sludge guideline (Snyman and Herselman, 2006) allows such quality sludges to be utilized in agriculture without restriction, as long as the N applied does not exceed crop demand, with the upper limit set at 10 Mg ha^{-1} yr^{-1}. Selected chemical characteristics of this class A1a sludge are presented in Table 3.

Table 3 Chemical characteristics of anaerobically digested, paddy dried sludge used during the 2004/05–2010/11 growing seasons (Source: Vlakplaas wastewater treatment plant).

Element	Unit	Year						
		2004/05	2005/06	2006/07	2007/08	2008/09	2009/10	2010/11
N	g kg^{-1}	30.3	18.8	22.2	30.9	27.4	22.4	27.5
P	g kg^{-1}	19.6	18.4	27.6	22.4	34.3	31.3	42.1
P-Bray1	mg kg^{-1}	166	154	40	66	50	80	120
Total C	g kg^{-1}	230	200	210	200	212	166	219.6
K	mg kg^{-1}	3804	710	689	1356	1720	2530	1850
Ca	mg kg^{-1}	25116	13062	17450	10042	26950	23147	28312
Mg	mg kg^{-1}	25116	13062	17450	10042	26950	23147	29312
pH	H_2O	6.01	6.2	6.02	6.08	8.1	5.7	5.6
Cd	mg kg^{-1}	1.63	0.07	0.15	18.9	19	12.2	10.75
Hg	mg kg^{-1}	1.70	0.02	0.03	1.81	1	1	0.4
Cr	mg kg^{-1}	51.93	1.50	2.92	503.8	419	369.7	315.8
As	mg kg^{-1}	7.08	0.18	0.23	17.94	6.5	5.88	5.64
Pb	mg kg^{-1}	54.46	19.41	61.37	102	75	88.4	66.67
Zn	mg kg^{-1}	459.9	40.3	200.8	2325	4920	3459	5755
Ni	mg kg^{-1}	23.83	10.37	50.97	144.50	152.00	99.30	103.27
Cu	mg kg^{-1}	97.2	3.21	4.59	526.8	681	497.29	544.52

Selection of metals

This study focuses on Zn, Cd, Ni and Pb because of their potential reactivity, toxicity, mobility and high natural background concentration in most South African soils, as well as high availability in local municipal sludges (Herselman et al., 2005). Cadmium is one of a very small group of metals which the Food and Agriculture Organization/World Health Organization have set a provisional daily intake limit for humans (70 µg Cd/day) (Berglund et al., 1983). Cadmium and lead are considered as the most mobile heavy metals (McLaughlin et al., 2000) and their concentration in plants is highly correlated with that found in soil (Pais and Benton, 1997).

Irrigation Scheduling

The irrigated maize-oat rotation experiment was planted under drip irrigation. The lateral spacing between dripper lines was 0.5 m from the summer 2005/06 season, but was reduced to 0.3 m after the 2005 winter season for the rest of the study period. Dripper spacing was 0.3 m in the laterals. The drip system was operated at a pressure of 100 to 150 kPa with an average drip rate of 13.8 mm hr^{-1}. In the absence of rainfall, maize was irrigated 10 mm every three days for the first four weeks after planting. In 2005, this was followed by irrigation according to the FAO crop factor method in the Soil Water Balance (SWB) model (Jovanovic and Annandale, 1999) once every five days until harvest in the absence of rainfall. During the growing seasons of 2007 to 2011, however, maize was irrigated according to neutron probe deficit readings to fill the profile to field capacity once a week in the absence of rainfall. During the winter seasons of each year, oats were irrigated every three days (9 mm in 2005 and 10 mm in 2006 and 2008) for the first four weeks. This was followed by irrigations to field capacity according to a site calibrated neutron water meter readings (Model 503 DR CPN Hydroprobe, Campbell Pacific Nuclear, California, USA) to a depth of 1.2 m for the rest of the season until harvest, mostly twice a week in 2005 and once a week during the rest of the study period.

Plant and Soil Sampling

In the 2010/11growing season, whole plant (above-ground) samples were collected for heavy metal uptake determination from an area of 0.5 m^2. Two plants per plot were taken under dryland maize and four under irrigated maize. A hand grab of additional plant samples were collected randomly from all plots during the 2010/2011 growing season for above-ground biomass determination.

After harvest, three soil samples were collected diagonally across each plot of a treatment at 0.3 m depth intervals down to 1.2 m using an auger. The samples were collected from the 0 to 0.3 m, 0.3 to 0.6 m, 0.6 to 0.9 m, and 0.9 to 1.2 m layers. The three samples from each layer of a plot were combined and mixed to make a single homogenous composite soil sample per layer (typically 48 soil samples were prepared for each cropping system).

Plant and Soil Chemical Analyses

Plant material was washed using distilled water to remove adhered soil particles and subsequently pulverized using a stainless steel shredder. All shredded maize plants (including stems, leaves, cobs and grains) were dried at 80°C for 72 hours and milled using a stainless steel mill. Plant samples (maize and oat stover, and maize grain) were analyzed for heavy metals after wet acid digestion using an inductively coupled plasma optical emission spectrometer (ICP-

OES) (SpectroFlame Modula; Spectro, Kleve, Germany), following standard procedures (Non-affiliated Soil Analyses Work Committee, 1990). Plant toxicity level was assessed based on the reference values provided by Jones (2012) (Table 4).

Table 4 General phytotoxic and acceptable levels of heavy metals in plant leaf tissue (dry mass) (Jones, 2012).

Heavy metal	Normal	Toxic	Excessive
	mg kg^{-1}		
Zn	10 - 100	100 - 150	150 – 400
Cd	0.05 - 0.20	0.20 - 5.0	5 – 30
Ni	0.50 - 10.0	10 - 50	50 – 100
Pb	5 – 30	30 - 100	100 – 300

Corresponding soil samples were air dried, pulverized and sieved using <2 mm sieves prior to acid digestion and analyzed using aqua regia (75% HCl, 25% HNO_3) for total metal concentration, and Ammonium Ethylenediaminetetraacetic acid (NH_4-EDTA) for available heavy metal concentration. Soil samples were also analyzed for pH (H_2O) and Carbon (C) using a Carlo Erba NA1500 C/N analyzer (Carlo ErbaStrumentazione, Milan, Italy). The threshold level of selected heavy metals in sludge amended soils was assessed as stipulated in the South African sludge guideline (Table 5), and guidelines for maximum permissible metal concentration in agricultural soils from other countries around the globe (Table 6). According to the current South African sludge guideline (Snyman and Herselman, 2006), the risk to the environment is unacceptable when the total metal content of the soil exceeds the total maximum threshold

(TMT) level. If the total metal content of the soil is between total investigative level (TIL) and the TMT (Table 5), the mobility of the metals concentration in the soil needs to be assessed.

Table 5 Metal limits for sludge amended soils (Snyman and Herselman, 2006).

Metal elements	**Total Investigative Level (TIL) (aqua regia)**	**Total Maximum Threshold (TMT) (aqua regia)**	**Maximum Available Threshold (MAT) (NH_4NO_3)**
	mg kg^{-1}		
Zn	185	200	5.0
Cd	2	3	0.1
Ni	50	150	1.2
Pb	56	100	3.5

Table 6 Guidelines for maximum permissible metal concentrations in agricultural soil across regions.

Heavy metal	**S.A. Sludge guideline[1]**	**Europe[2]**	**USA[3]**	**Australia & New Zealand[2]**
		mg kg^{-1}		
Zn	200	150-300	1400	200
Cd	3	1-3	20	3
Ni	150	30-75	210	60
Pb	100	50-300	150	300

[1]Snyman and Herselman (2006) [2]McLaughlin et al. (2000) [3]US EPA (1995)

Total investigate level (TIL) is a flag that warrants detailed soil analyses in terms of mobility using NH_4NO_3 extraction. If the NH_4NO_3 extractable metal content of the soil exceeds the maximum available threshold (MAT), sludges of pollutant class B may not be applied to this soil. If the NH_4NO_3 extractable metal content of the soil is lower than the MAT, sludge of pollutant class B can be applied to the soil and the land should be re-assessed two years after sludge application (Snyman and Herselman, 2006). The soluble fraction of soil heavy metals was also analyzed using saturated paste extracts. The saturated pastes were prepared by adding deionized water to 250 g of air-dried samples until it reached a condition of complete saturation, as described by the standard procedures in Non-affiliated Soil Analyses Work Committee (1990). Saturated pastes were allowed to equilibrate for 24 hours in a covered container. An extract from the saturated paste was acquired by filtering the soil paste through Whatman no 50 paper on a Buchner funnel under low suction (20 kPa).

Statistical Analyses

Statistical analyses were performed to evaluate the effect of varying sludge application rates on heavy metal uptake by dryland maize, irrigated maize and oat, and concentrations in the soil. The statistical analyses were conducted using Analysis of Variance (ANOVA) and General Linear Model (GLM) procedures of Windows SAS Version 9.3 (SAS Institute, 2010) to determine significant treatment effects on measured response variables. When treatment effects were found to be significant, Fisher's protected LSD test at the 0.05 level was used to separate means.

Result and Discussion

Heavy Metal Uptake by Crops

Zinc (Zn)

Zinc uptake by both dryland maize and the irrigated maize oat-rotation increased significantly as sludge application rate increased (Fig. 1a). This is in agreement with previous findings by Hinesly et al. (1978), who reported a significant increase in Zn uptake by maize (20 inbred lines) as sludge application rate increased. Generally Zn uptake was significantly lower for the irrigated maize than for dryland maize treatments receiving similar levels of sludge, except for the 16 Mg ha^{-1} yr^{-1} treatment, where uptake by dryland maize was relatively higher, although not significantly so. Nonetheless, the annual Zn uptake by the irrigated maize-oat rotation from sludge amended treatments was significantly higher than similar dryland maize treatments. The lower Zn uptake by the irrigated maize is mainly attributed to the reduction in Zn plant availability because of the extra Zn removal by oats (Fig. 1b) during the winter season. The amount of Zn taken up by oats was generally lower than similar irrigated maize treatments (Fig. 1b) despite receiving similar Zn amounts. Such variations in Zn uptake between crops and within cultivars of a specific crop are common (Hinesly et al., 1978; Alloway, 1995).

It was also apparent that Zn stored in the stover (stems and leaves) was significantly higher than that in the grain (Fig. 1b). Previous studies conducted by Hinesly et al. (1978) and Granato et al. (2004) also report similar results. Grain Zn storage accounted for 13 to 19%, 30 to 33%, and 22 to 27% of the total crop aboveground biomass Zn uptake from the 0, 8 and 16 Mg ha^{-1} yr^{-1} sludge treatments, respectively, by dryland and irrigated maize-oat rotation. The fraction of Zn stored in maize grain from the 8 Mg ha^{-1} yr^{-1} and 16 Mg ha^{-1} yr^{-1} treatments were similar to the low-medium (33 – 36%), and high (26%) sludge application treatments of Bidwell and Dowdy (1987), respectively. Similarly, the fraction of Zn in maize grain from the 8 Mg ha^{-1} yr^{-1} sludge treatment was closer to the findings of Shivay and Prasad (2014) (34 – 39%) for maize that received Zn as micro nutrient through foliar and soil applications. The fraction of Zn in maize grain for the control treatment from this study was much lower than the 63% of Bidwell and Dowdy (1987), as well as, the 37% of Shivay and Prasad (2014). This is most probably due to the lower natural background concentration of Zn from this study (0.0155 mg kg^{-1}; total) compared with that of Bidwell and Dowdy (1987) (9.10 mg kg^{-1}; total) as well as Shivay and Prasad (2014) (0.36 mg kg^{-1}; plant available).

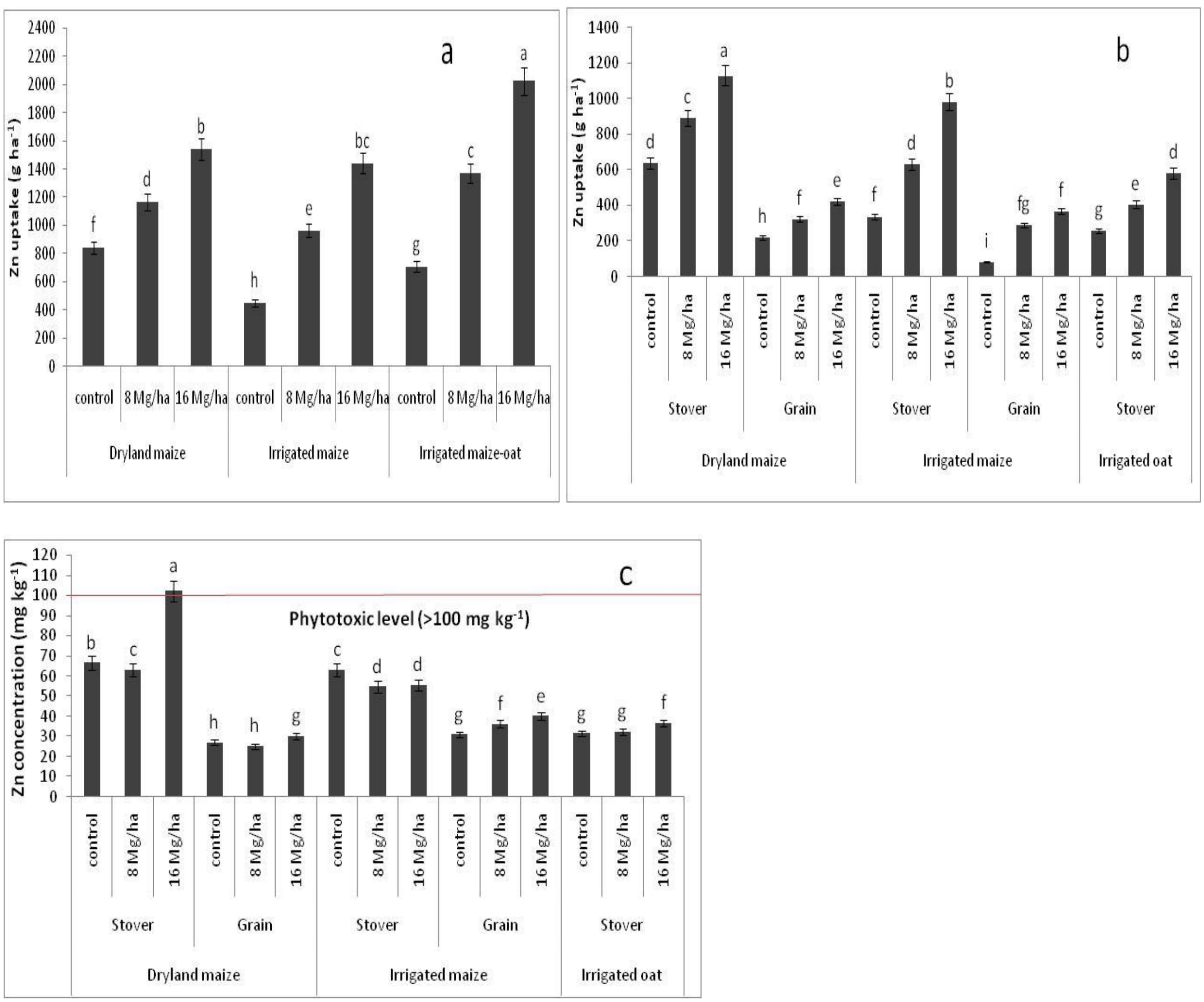

Fig.1. Total aboveground biomass zinc uptake by dryland maize, irrigated maize and irrigated maize-oat rotation (a), and zinc uptake by maize stover vs. grain (b) as well as concentration of zinc in stover and grain of dryland maize and irrigated maize-oat rotation (c) planted to a clay loam Hutton soil treated with municipal sludge at varying rates for seven years.

The low grain Zn concentration is an indication of low Zn transfer from vegetative biomass to the grain (Fig. 1b). Similar to the patterns of total Zn uptake, Zn uptake by stover and grain increased as the sludge application was doubled under both cropping systems but was only significant under dryland (Fig. 1b). It was interesting to note that the concentration of Zn in maize stover (Fig. 1c) under dryland production, reached phytotoxic levels (103 mg kg^{-1}) for the 16 Mg ha^{-1} yr^{-1} sludge treatment following the reference values provided by Jones (2012) in Table 4. This is in contrast to soil Zn concentration (95 mg kg^{-1} near the soil surface) which was far below environmental threshold levels (185 mg kg^{-1}) stipulated by South African sludge guideline (Table 5) as well as other international guidelines for maximum permissible metal concentration in agricultural soils (Table 6). The high dryland maize Zn concentration under the 16 Mg ha^{-1} yr^{-1} application rate, is most probably attributed to a combination of factors, including relatively low biomass production (Tesfamariam, 2009) and therefore lower crop Zn uptake over the 7 consecutive years of this study compared with the irrigated system, which might have resulted in the buildup of Zn in the soil. This is in agreement with previous findings who reported high Zn uptake at high sludge application rates because of Zn accumulation in the soil (Miller et al., 1995; Kalbitz and Wennrich, 1998; and Merrington et al., 2003). The high Zn uptake observed at high sludge application rates under dryland maize relative to equivalent irrigated maize-oat rotation is mainly attributed to the increase in the amount of Zn added to the soil with the sludge. The observed reduction in soil pH as the sludge application rate increased

(Table 7) could have also contributed to such increase in Zn uptake. This is in agreement with previous findings of Miller et al. (1995), who reported that, Zn uptake is mainly associated with high metal concentrations in sludges, and/or low soil pH values of 4.5 – 6.

Table 7 Soil pH (H_2O) and organic C (%) in the soil profile of both dryland maize and irrigated maize-oat rotation for the growing season of 2010/11.

		Soil pH (H_2O)			**Soil C (%)**		
		Sludge application rates			**Sludge application rates**		
		Mg ha^{-1}					
Cropping system	**Soil depth (m)**	**0**	**8**	**16**	**0**	**8**	**16**
	0 – 0.3	6.54	5.68	5.44	1.37	1.52	1.58
	0.3 – 0.6	6.27	5.76	6.07	0.8	0.94	0.81
	0.6 – 0.9	5.89	5.90	5.81	0.39	0.48	0.53
Dryland maize	0.9 – 1.2	5.81	5.78	5.44	0.28	0.39	0.31
	0 – 0.3	7.1	6.83	6.00	1.4	1.54	1.64
	0.3 – 0.6	6.47	6.72	6.60	0.92	0.94	0.77
	0.6 – 0.9	6.4	6.44	6.53	0.46	0.58	0.43
Irrigated maize-oat rotation	0.9 – 1.2	6.07	6.03	6.16	0.33	0.42	0.27

The increasing trend in Zn uptake observed in this study strengthens concerns raised by Lotter and Pitman (1997), who point out that uncontrolled utilization of sewage sludge on agricultural lands will lead to accumulation of Zn in the receiving soil, which could lead to a permanent risk to plants, and thereby compromise sustainability.

Cadmium (Cd)

Cadmium uptake by dryland maize and irrigated maize-oats also increased significantly as sludge application rate increased (Fig. 2a). Generally, Cd uptake by irrigated maize was higher than for dryland maize treatments (Fig. 2a). This is in contrast to Zn uptake, which was relatively lower for equivalent irrigated maize treatments. This indicates that the mobility of Cd from soil to crop is influenced by the availability of soil water. This leads to the acceptance of hypothesis 2 "Uptake of heavy metals under irrigated maize-oat rotation is higher than dryland maize" for Cd. This supports previous findings that the mobility of Cd in the soil-plant system is greater than for Zn (Sauerbeck, 1991; McLaughlin et al., 2000; Legind et al., 2012). Similar to Zn, the amount of Cd stored in stover was significantly higher than the grain under both cropping systems (Fig. 2b). It was also interesting to note that Cd uptake by stover and grain significantly increased as the sludge application rate increased. This is attributed to the increase in Cd concentration in the soil at higher sludge levels and is highly correlated to plant uptake (Pais and Benton, 1997). Cadmium in the grain of both cropping systems accounted for 38 to 45% of the total aboveground biomass Cd uptake, indicating the mobility of Cd within the plant system. This is in contrast to the 13 to 33% for Zinc. The fraction of Cd in maize grain relative to the total aboveground biomass Cd uptake from this study was higher than the 3-12% of Bidwell and Dowdy (1987). Such big differences could most probably be because of the genetic differences between the cultivars (Hinesly et al., 1978; Alloway, 1995) among other environmental factors.

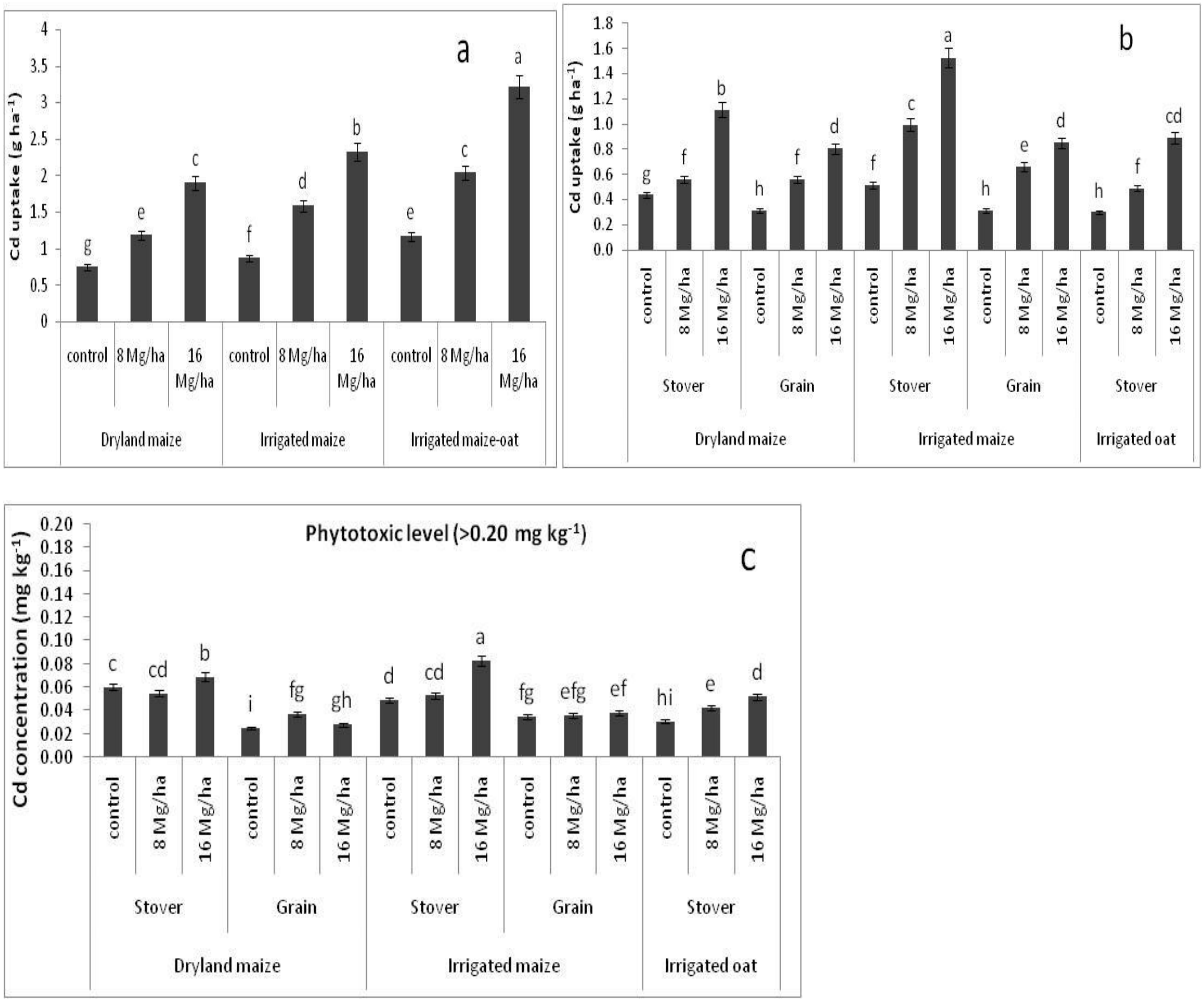

Fig.2. Total aboveground biomass cadmium uptake by dryland maize, irrigated maize and irrigated maize-oat rotation (a), and cadmium uptake by maize stover vs. grain (b) as well as concentration of cadmium in stover and grain of dryland maize and irrigated maize-oat rotation (c) planted to a clay loam Hutton soil treated with municipal sludge at varying rates for seven years.

The observed increase in Cd uptake by crops as the sludge application rate increased is mainly attributed to increase in the amount of Cd added to soil. The reduction in soil pH at higher sludge application rate (Table 7) could have also contributed to the increase in Cd uptake. This is because the bioavailability of Cd and metals in general increases as the soil pH decreases below 7 (Kalbitz and Wennrich, 1998). Despite the highly mobile nature of Cd, the concentration within the plant tissue remained below phytotoxic levels (>0.2 mg kg^{-1}) (Fig. 2c) following seven years of continuous sludge application at varying rates.

Nickel (Ni)

Nickel uptake by both cropping systems increased significantly as the sludge application rate increased (Fig. 3a). This is mainly attributed to the increase in the amount of Ni added to the soil. The reduction in soil pH as the sludge application rate increased (Table 7) could have also contributed to such increase in Ni uptake because bioavailability of metals increases as soil pH decreases (Kalbitz and Wennrich, 1998). Nickel uptake by irrigated maize was significantly higher than for dryland maize treatments (Fig. 3a). This is despite the application of only half of the annual sludge application at planting of the irrigated maize, in contrast to the full application for the equivalent dryland maize treatments. Annual Ni uptake of 16 Mg ha^{-1} yr^{-1} under irrigated maize-oat rotation was double that of similar dryland maize treatments. This indicates that Ni is quite soluble and its uptake by maize is influenced by the availability of water. Therefore, hypothesis 2 “Uptake of heavy metals under irrigated maize-oat rotation is higher than dryland maize” was accepted for Ni.

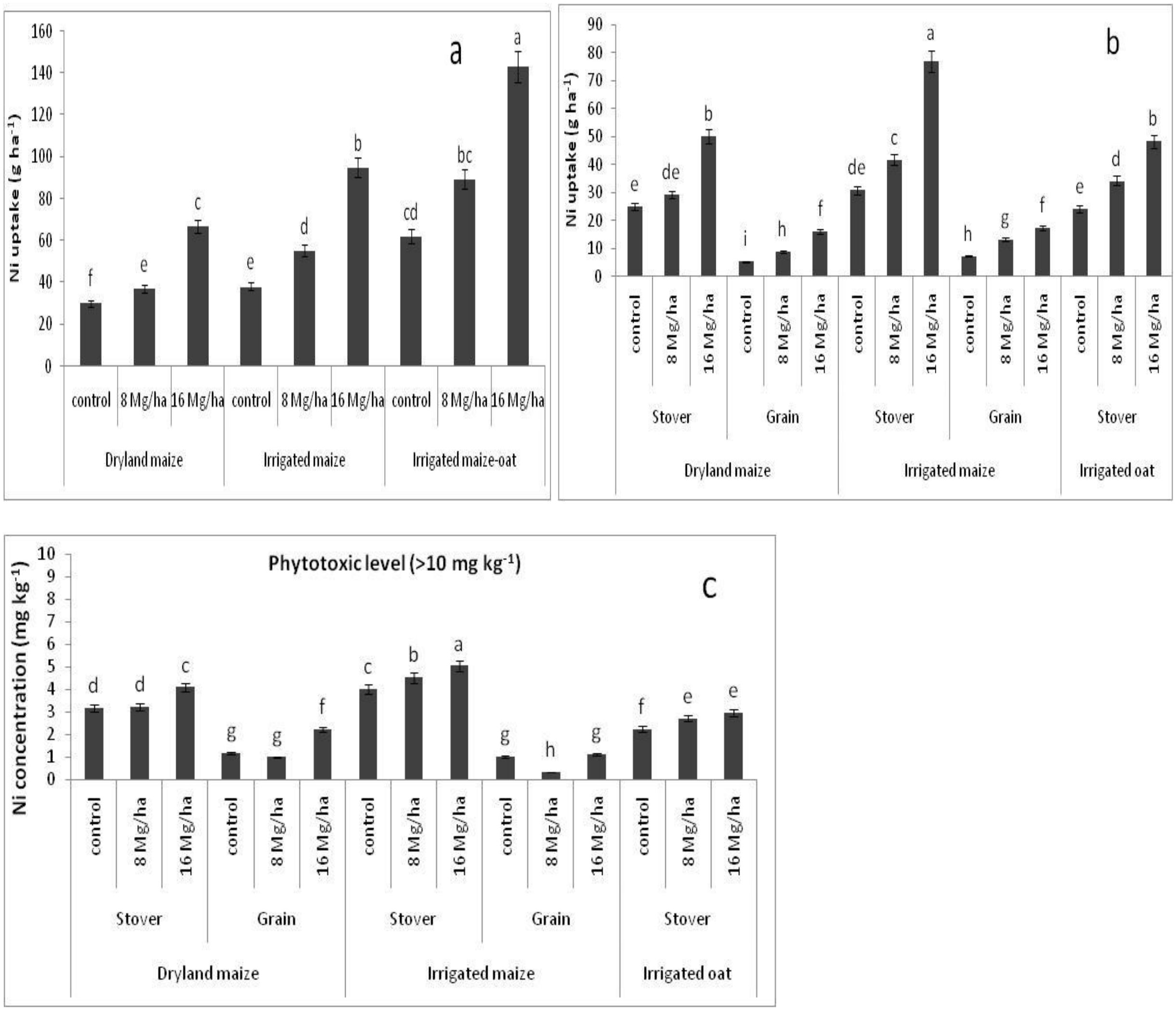

Fig.3. Total crop nickel uptake by dryland maize, irrigated maize and irrigated maize-oat rotation (a), and nickel uptake by maize stover vs. grain (b) as well as concentration of nickel in stover and grain of dryland maize and irrigated maize-oat rotation (c) planted to a clay loam Hutton soil treated with municipal sludge at varying rates for seven years.

Similar to Zn and Cd, the amount of Ni stored in stover was significantly higher than that in the grain under both cropping systems (Fig. 3b). Nickel uptake by stover and grain increased significantly as the sludge application rate increased, except for stover under the 8 Mg ha^{-1} yr^{-1} dryland maize, which was not significantly different compared with the zero control. The concentrations in the plant tissue, however, remained below phytotoxic levels (>10 mg kg^{-1}) (Fig. 3c). Nickel concentration in the grain accounted for 14 to 22% of uptake at lower sludge application rates of 0 and 8 Mg ha^{-1} yr^{-1}, and 9 to 14% at 16 Mg sludge ha^{-1} yr^{-1}. This indicates that Ni has relatively lower mobility within the plant system than Zn and Cd.

Lead (Pb)

Crop uptake of Pb increased significantly as sludge application rate increased (Fig. 4a). Similar to Zn, Cd and Ni, this is mainly attributed to the increase in the amount of Pb added to the soil with the sludge. The reduction in soil pH as the sludge application rate increased (Table 7) could have also contributed to such increase in Pb uptake because heavy metal availability increases as soil pH drops below 7 (Kalbitz and Wennrich, 1998). It was interesting to note that the annual aboveground biomass Pb uptake by irrigated maize was significantly higher than for dryland maize treatments receiving the same amount of sludge. This is despite the application of only half the annual sludge application at planting of the irrigated maize, in contrast to the full application for dryland maize. The annual crop Pb uptake by the irrigated maize-oat rotation was more than double that of dryland maize. Similar to Ni, the uptake of Pb by maize was influenced by the availability of water. Thus hypothesis 2 was also accepted for Pb. Generally, Pb uptake by stover was significantly higher than grain uptake, both for dryland maize and the irrigated maize-oat rotation (Fig. 4b). Similarly, both stover and grain Pb uptake increased significantly as the sludge application rate increased for both cropping systems.

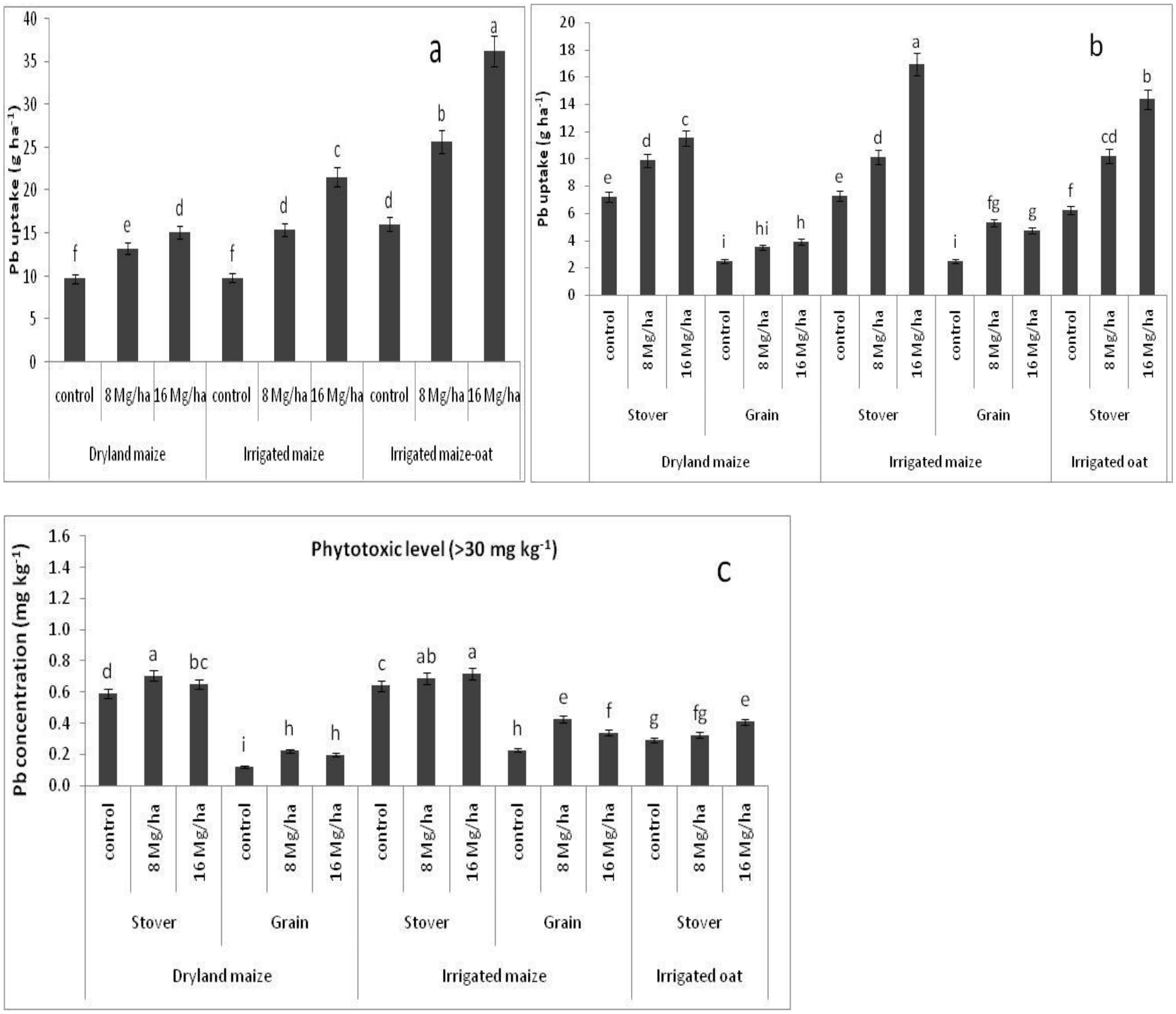

Fig.4. Total crop lead uptake by dryland maize, irrigated maize and irrigated maize-oat rotation (a), and lead uptake by maize stover vs. grain (b) as well as concentration of lead in stover and grain of dryland maize and irrigated maize-oat rotation (c) planted to a clay loam Hutton soil treated with municipal sludge at varying rates for seven years.

The amount of Pb in the grain accounted for 20 to 29% of the total Pb stored in the aboveground biomass for the treatments that received sludge rates of 8 and 16 Mg ha^{-1} yr^{-1}. This indicates that Pb has relatively moderate mobility within the plant system. The parallel increase in Pb uptake as the sludge application rate increased is a good indication of the potential accumulation that could lead to phytotoxicity. Nonetheless, the concentration of Pb in plant tissues following 7 years of municipal sludge application was far below phytotoxic levels (>30 mg kg^{-1}) (Fig. 4c).

Heavy Metal Accumulation and Mobility in the Root Zone

Zinc (Zn)

The mean soil profile Zn concentration under dryland maize increased significantly as sludge application rate increased (Fig. 5a). Under irrigated maize-oat, however, the mean soil profile concentration of the 16 Mg ha^{-1} yr^{-1} was lower than that of 8 Mg ha^{-1} yr^{-1}. This is most probably because of a higher biomass production from the 16 Mg ha^{-1} yr^{-1} (20% higher) than for the 8 Mg ha^{-1} yr^{-1} treatment (Tesfamariam, 2009) during the past six years, which might have resulted in higher plant uptake. Generally there was no clear pattern of Zn translocation (leaching) to deeper layers as a function of sludge application rate, but the general pattern seemed to follow a sinusoidal curve, except for the irrigated 16 Mg ha^{-1} yr^{-1} treatment, which had a sigmoidal pattern with high concentrations in the top 0.3 m layer (Fig. 5b). Nonetheless, there is a clear indication of Zn translocation to lower layers, which increased as sludge application rate increased, except for the irrigated 16 Mg ha^{-1} yr^{-1} treatment. This is in agreement with previous findings of Hinesly et al. (1978); Boswell (1975); and Sidle and Kardos (1977), who all report the movement of Zn below the depth of incorporation in agricultural lands. In contrast, Ippolito and Barbarick (2008) did not find Zn mobility below 20 cm under dryland wheat production on a plat-ner loam soil, which received sludge at rates of 0, 6.7, 13.4, 26.8 and 40.3 dry Mg ha^{-1} every 2 years from 1982 to 2002. The absence of Zn mobility below 20 cm in the

Ippolito and Barbarick (2008) study, is most probably attributed to the low rainfall of the study site (350 mm) compared with our study site, which has a long-term annual rainfall of 700 mm. It was apparent that Zn distribution in the soil profile (Fig. 5a) seems to have similar patterns to that of soil organic matter (Table 7), which agrees with the findings of Antoniadis and Alloway (2002), who report a direct relationship between organic matter and Zn concentration in sludge amended soils.

The overall concentration of Zn in the soil profile of all treatments was still far below the total investigative level (185 mg kg^{-1}) of the South African sludge guideline (Table 5) and international guidelines for maximum permissible metal concentration in agricultural soils (Table 6). However, the increase in Zn concentration by 250% following 7 years of 16 Mg ha^{-1} yr^{-1} sludge applications indicate the potential for future build up. A large fraction of the total Zn for both dryland maize and irrigated maize-oat rotation was EDTA extractable or plant available. The water soluble fraction was, however, well below 1% of the total concentration throughout the profile, indicating that water soluble Zn was not the main contributor to the translocation of heavy metals. Therefore hypothesis 1, “Continuous use of good quality sludge with low heavy metal content will not result in a significant accumulation of water soluble heavy metal fraction in the short term” was accepted.

Cadmium (Cd)

The mean Cd concentration in the soil profile of both dryland maize and the irrigated maize-oat rotation system, increased significantly as the sludge application rate increased (Fig. 5c). Similar findings were reported by Baveye et al. (1999) on a liquid sludge treated silt loam soil, where the total concentration of Cd increased as sludge application rate increased. There was, however, no significant difference between dryland maize and irrigated maize-oats that received the same sludge application rates. It was also interesting to note that most of the Cd for the 16 Mg ha^{-1} yr^{-1} dryland maize and irrigated maize-oat rotation accumulated in the top 60 cm layer and decreased with depth (Fig. 5d). This agrees with findings of Baveye et al. (1999), who report Cd mobility to a depth of 75 cm, which also decreased with depth. In contrast, relatively high concentrations of Cd accumulated down to 1 m for the 8 Mg ha^{-1} yr^{-1} of both cropping systems. The distribution of Cd in the top 0.3 m layer and 0.9-1.2 m soil layer (Fig. 5d) followed similar patterns to soil organic C and pH (H_2O) of the corresponding layers (Table 7). This too is in agreement with the findings of Antoniadis and Alloway (2002), who report an increase in the mobility and plant availability of Cd at high sludge application rates because of the increase in dissolved organic matter.

The concentration of Cd in the whole profile of both cropping systems, however, was far below the total investigative level (2 mg kg^{-1}) of the South African sludge guideline (Table 5), guidelines from other regions of the world (1-20 mg kg^{-1}) (Table 6), and the global soil mean Cd concentration of 0.6-1.1 mg kg^{-1} (Kabata-Pendias and Pendias, 2000). The EDTA extractable and saturated paste extractable (mobile) Cd fraction in the soil profile of both cropping systems was below the method detection limit (<1 mg kg^{-1}). This is in contrast to the findings of Baveye et al. (1999), who reported an increase in the DTPA extractable Cd in the top 45 cm soil layer. This indicates that the excess accumulated Cd was complexed either with soil minerals or organic matter within the soil. Therefore, hypothesis 1 was accepted for both cropping systems.

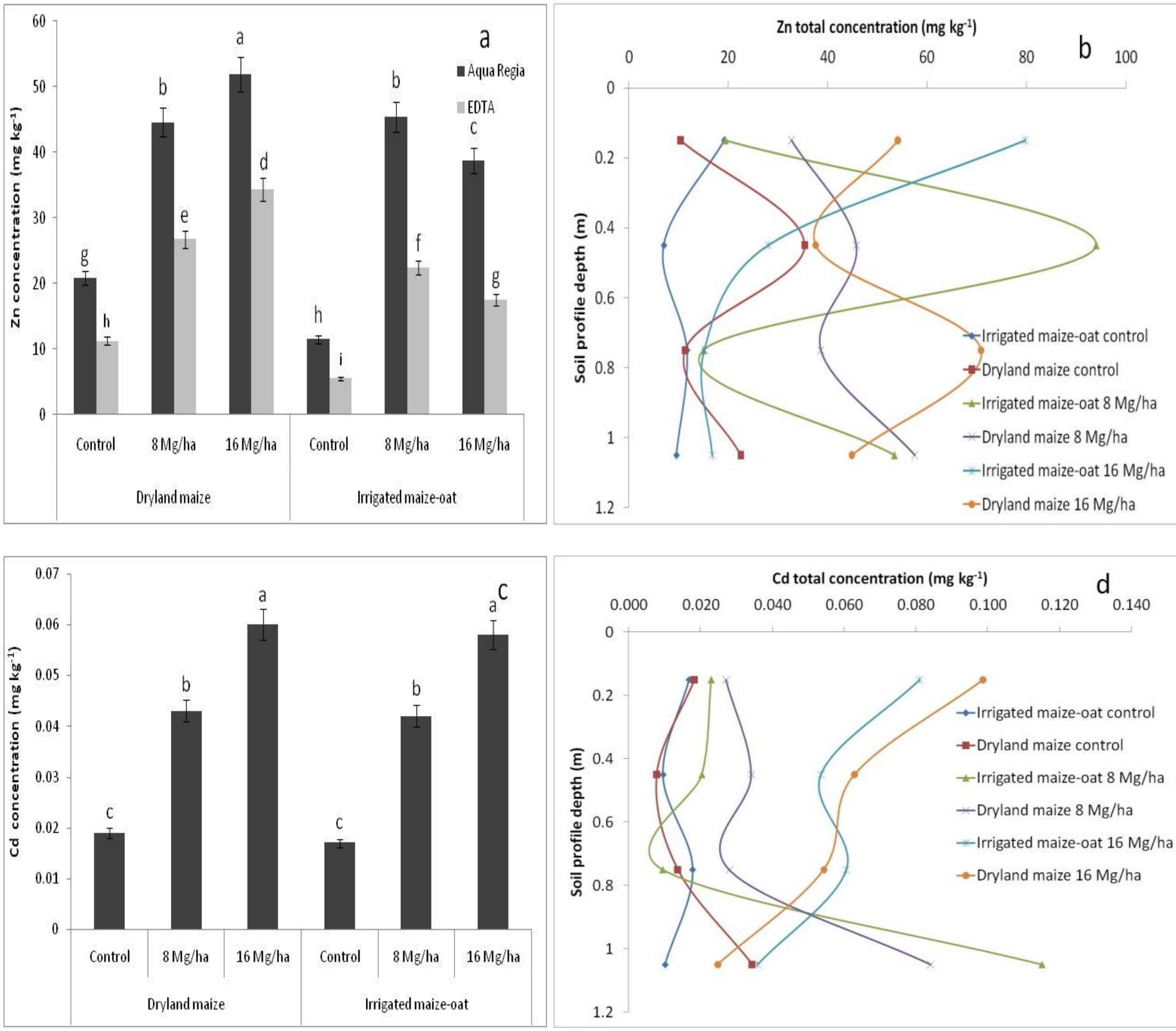

Fig.5. Mean soil profile total (Aqua Regia extractable) and plant available (Ethylenediaminietetraacetic acid extractable) concentrations of zinc (a), and cadmium (c) as well as mean soil profile total (Aqua Regia extractable) concentrations of zinc (b) and cadmium (d), distribution in the top 1.2 m layer of a clay loam Hutton soil treated with class A1a sludge for seven years (Plant available (Ethylenediaminietetraacetic acid extractable) fraction of Cadmium was below the method detection limit <1 mg kg^{-1}).

Nickel (Ni)

Nickel accumulation in the soil profile did not increase with doubling of the 8 Mg ha^{-1} yr^{-1} norm, both under dryland maize and in the irrigated maize-oat rotation (Fig. 6a). This is in contrast with the patterns observed for Zn and Cd. It was also interesting to note that the mean soil profile Ni concentration of dryland maize was significantly higher than the same irrigated treatments. This is most probably because of higher plant uptake (Fig. 3a) and potential leaching below 1.2 m under irrigation because Ni in sewage sludge is mainly in soluble form and is readily available for plant uptake as well as leaching (Kabata-Pendias and Pendias, 2000). The concentration of Ni in the whole profile of both cropping systems, however, is far below the total investigative level (50 mg kg^{-1}) of the South African sludge guideline (Table 5) as well as other international guidelines (Table 6).

The increasing Ni concentration towards the bottom of the soil profile (Fig. 6b), under the irrigated 8 and 16 Mg ha^{-1} yr^{-1} treatments provides evidence for possible leaching below the point of measurement (1.2 m) because of the mobile nature of Ni as reported by Snyman and Van Der Waals (2004). According to Snyman and Van Der Waals (2004) heavy metals such as Ni leached 8 cm below the plough layer in a short time and suggest that there is a risk of these metals moving below the incorporation zone. Unlike Zn and Cd, water-soluble Ni was detected at low concentration levels (≤1% of the EDTA extractable fraction). Therefore, hypothesis 1 was accepted for Ni. In addition, considering the low soil profile Ni concentration and the increase in Ni concentration with depth under irrigated conditions, hypothesis 2 was also accepted for Ni.

Lead (Pb)

Generally the mean Pb concentration in the soil profile of dryland maize was significantly higher than under irrigation, except for the 16 Mg ha^{-1} yr^{-1} sludge treatment (Fig. 6c). Under irrigated system soil profile Pb concentration increased significantly as sludge application rate increased, which was not the case for the dryland system. A large fraction of the Pb in the soil profile (46 to 79%) was plant-available (EDTA extractable), but the water-soluble fraction was below the method detection limit (<1 mg kg^{-1}). Consequently, hypothesis 1 was accepted for both dryland and irrigated maize-oat rotation.

Lead concentration in the soil profile of the zero control treatment remained the lowest throughout the profile (Fig. 6d), while the irrigated as well as dryland maize production systems that received 16 Mg ha^{-1} yr^{-1} sludge annually showed an increase in Pb concentration below 0.6 m. This indicates the potential leaching losses that might have occurred during the study period and could probably be the reason for the reported insignificant difference in soil profile concentration between the 8 and 16 Mg ha^{-1} yr^{-1} treatments under dryland maize production. The distribution of Pb in the top 0.3 m of the soil profile and between 0.6-0.9 m (Fig. 6d) followed similar patterns to soil organic C (Table 7). Previous studies conducted by Baveye et al. (1999) also report significant mobility of Pb in their case, to a depth of 45 cm. The overall concentration of Pb is, however, below the total investigative level (56 mg kg^{-1}) as stipulated in the South African sludge guideline (Table 5) as well as those from other countries (Table 6).

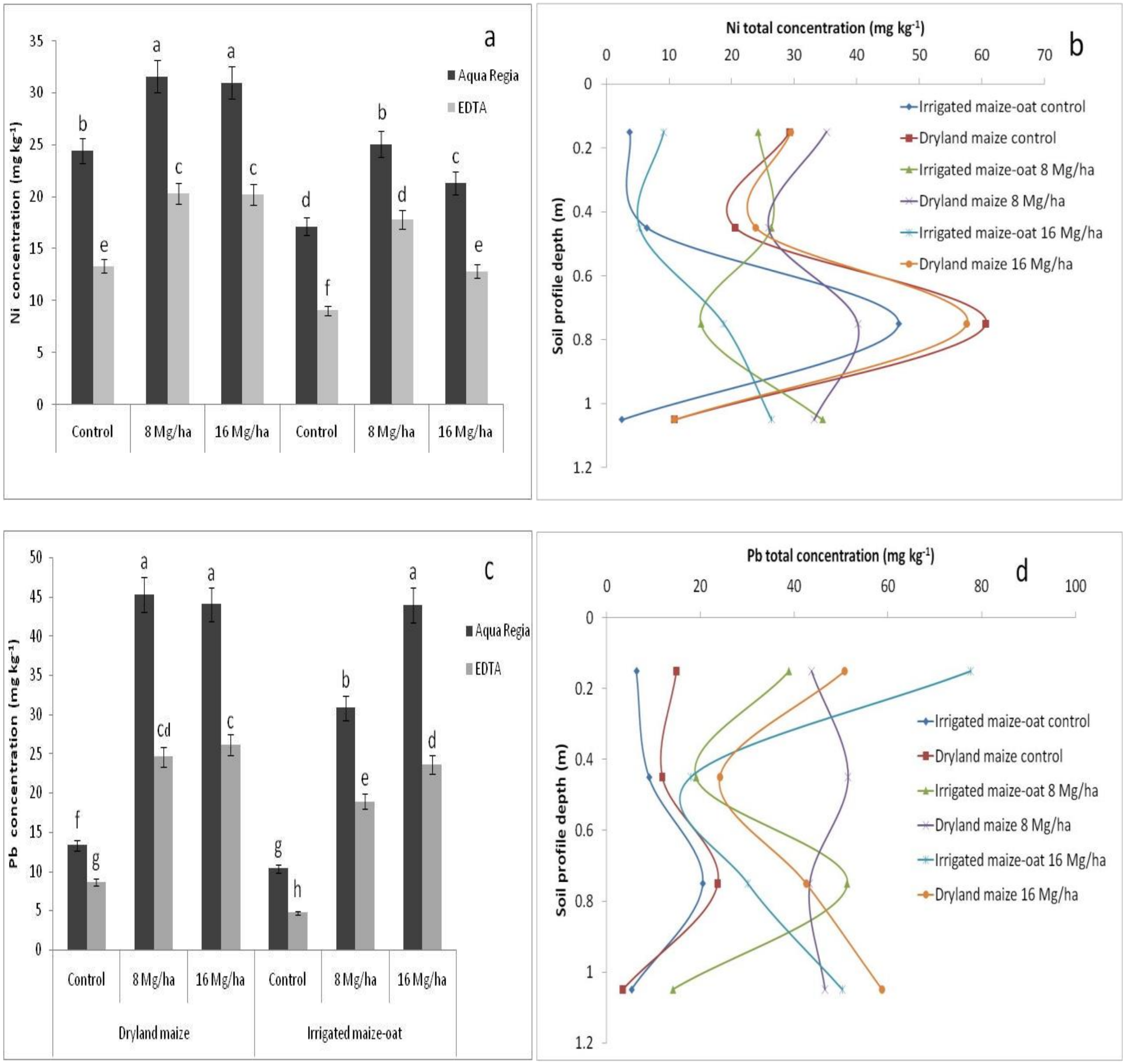

Fig.6. Mean soil profile total (Aqua Regia extractable) and plant available (Ethylenediaminietetraacetic acid extractable) concentrations of nickel (a), and lead (c) as well as mean soil profile total (Aqua Regia extractable) nickel (b) and lead (d) distribution in the top 1.2 m soil profile of a clay loam Hutton soil treated with class A1a sludge for seven years.

The mean soil profile Pb concentration of the 16 Mg ha^{-1} yr^{-1} sludge treatments for dryland maize and irrigated maize-oat rotation increased by 30.56 and 32.06 mg kg^{-1}, respectively after 7 consecutive years of sludge application. Assuming negligible leaching and constant sludge heavy metal content, annual sludge application rate of 16 Mg ha^{-1} yr^{-1} could raise soil Zn concentration of the study site to total maximum threshold levels after 20, 30 and 145 years, according to the South African (Snyman and Herselman, 2006), European (McLaughlin et al., 2000), and USA (US EPA, 1995) guidelines, respectively. Similar application rates would raise soil Cd concentration to TMT levels after 86 years according to South African and European guidelines, and 580 years according to USA guideline. Nickel could reach to TMT levels after 370, 140 and 550 years, and Pb after 330, 1100 and 525 years according to the South African, European, and USA guidelines, respectively. Nevertheless, the potential for leaching observed from this study warrants monitoring protocols that take into account pollutant distribution within a soil profile over time.

Heavy Metal Mass Balance (Supply less Uptake Mass Balance)

Heavy metal mass balance based on sludge input less crop output is not the full story because there are other sources and sinks for these metals, in particular losses through leaching. Mass balance was calculated by subtracting the metal exported (crop uptake) from the total applied in sludge.

According to a supply less uptake mass balance of a single year (2010/11), Zn uptake by crops accounted for only 3 to 5% of what was added with sludge (Table 8). This indicates that more than 95% of Zn added with sludge should accumulate in the soil profile. This is in agreement with findings of Chang et al. (1984), who report a significant accumulation of Zn in the soil profile of sludge amended soils. The negative mass balance of the zero control treatment indicates that the crop used Zn from the soil reserve. Similar to Zn, based on the mass balance of Cd applied with sludge less that removed by crop, only 3 to 6% of what was added with the sludge was taken up by the crop under both cropping systems (Table 8). This was the reason for the significant buildup of Cd observed in the soil profile as the sludge application rate was doubled (Fig. 5c).

Table 8 Mass balances of Zn, Cd, Ni and Pb under dryland maize and irrigated maize-oat rotation for the growing season of 2010/11.

Heavy metal	**Sludge application Rate**	**Metal Supply**	**Metal Uptake**		**Metal Supply less Uptake**	
			Dryland	**Irrigated**	**Dryland**	**Irrigated**
	Mg ha^{-1}	kg ha^{-1}				
Zn	0	0	0.8482	0.4101	-0.8482	-0.4101
	8	46.04	1.1969	0.9049	44.843	45.135
	16	92.08	1.4861	1.4012	90.593	90.678
Cd	0	0	0.0005	0.0012	-0.0005	-0.0012
	8	0.086	0.0016	0.0017	0.0844	0.0843
	16	0.172	0.0018	0.0029	0.1702	0.1691
Ni	0	0	0.0279	0.0200	-0.0279	-0.0200
	8	0.826	0.0395	0.0370	0.7865	0.789
	16	1.652	0.0788	0.0970	1.5732	1.555
Pb	0	0	0.0085	0.0101	-0.0085	-0.0101
	8	0.533	0.0128	0.0141	0.5202	0.5189
	16	1.067	0.0158	0.0226	1.0512	1.0444

Based on the mass balance of Ni added with sludge less uptake (removed) by crop, there was a net positive Ni accumulation in the profile of both dryland maize and irrigated maize-oat rotation (Table 8). Of the total Ni added within a year, only 4.5 to 5.5% of the Ni was recovered by the plant. The rest either accumulated in the soil profile or leached below the depth of measurement. Similar to Zn and Cd, the net positive Ni mass balance highlights future potential accumulation in the soil profile and warrants setting monitoring protocols.

Based on the mass balance of Pb added with sludge less uptake by dryland maize and irrigated maize-oats, sludge application resulted in a net positive mass balance indicating a potential build up of Pb in the soil though at a low rate (Table 8). According to this study, only 2 to 5% of the total Pb added to the soil was taken up by the plant with, the rest mostly accumulating in the soil profile. This provides evidence for possible accumulation or binding of Pb in sludge-amended soils as reported by Planquart et al. (1999). According to Planquart et al. (1999) most of the Pb in sludge amended soil was found as an organic matter bound fraction.

Therefore, it is of utmost importance to have integrated management and monitoring protocols for beneficial agricultural use of sludge in order to minimize potential long-term risks to human health and the environment in general.

Conclusion

Crop uptake and accumulation in the soil profile of Zn, Cd, Ni and Pb increased as the sludge application rate increased. Concentrations in tissues of the test crop remained well below phytotoxic levels, except for Zn under dryland maize production that received sludge at 16 Mg ha^{-1} yr^{-1}. Concentrations of the selected pollutants in the soil profile of all sludge treatments remained below threshold levels as stipulated in the South African sludge guideline as well as international guidelines. A large fraction of these metals was EDTA extractable. The saturated paste extractable fractions of Cd and Pb were <1 mg kg^{-1}. However, water soluble fractions of Zn and Ni were detected though <1% of the EDTA extractable fraction, indicating the mobile nature of these elements and potential for leaching and groundwater contamination. Consequently, hypotheses 1 and 3 were accepted for the metals studied and hypothesis 2 was rejected for Zn. Therefore, it is of utmost importance to have integrated sludge management practices and rigorous heavy metal monitoring protocols below the top 0.3 m plough layer for sustainable beneficial agricultural use of sludge. Further investigation on metal leaching below the active root zone is recommended.

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