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Source: Canadian Journal of Soil Science, 101(2) : 305-316

Published By: Canadian Science Publishing

URL: <https://doi.org/10.1139/cjss-2020-0124>

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# Cattle manure loadings and legacy effects on copper and zinc availability under rainfed and irrigated conditions

Srimathie P. Indraratne, Matthew Spengler, and Xiyang Hao

**Abstract:** Long-term cattle manure applications build up nutrient pools and can lead to trace element enrichments in soils. The objectives of this study were to evaluate copper (Cu) and zinc (Zn) loadings in the soil during continuous annual cattle manure applications and determine the time required for soil to return to its pre-manure available Cu and Zn levels after manure is discontinued. The manure application rates were 0, 30, 60, and 90 Mg·ha<sup>-1</sup> for rainfed and 0, 60, 120, and 180 Mg·ha<sup>-1</sup> (wet weight) for irrigated plots. Although manure was applied for 45 yr in some plots, applications were terminated in one subset of treatments after 14 yr and in another subset after 30 yr to study legacy effects after 31 and 15 yr, respectively. Soil samples were collected in the fall of 2003, 2008, 2013, and 2018 and analyzed for available Cu and Zn. Crops were grown in all years continuously with Cu and Zn concentrations measured in both silage and grains harvested. The regression model developed using data collected suggests long legacy effects with recovery time to pre-manure levels ranging from 10 to 20 yr for Cu and 23 to 41 yr for Zn at irrigated and 10–24 for Cu and 21–32 yr for Zn under rainfed, respectively. Long-term applications of cattle manure could lead to accumulation of Cu and Zn, creating long-lasting legacy effects in soils with the increased environmental risk of leaching to groundwater.

*Key words:* cattle manure, Cu and Zn loadings, legacy effect, long-term field experiments, manure application rates.

**Résumé :** L'application prolongée de fumier de bovins entraîne une accumulation d'oligoéléments et peut conduire à l'enrichissement du sol par les éléments à l'état de traces. Les auteurs voulaient évaluer la charge de Cu et de Zn dans le sol, consécutivement à des applications annuelles continues de fumier de bovins et déterminer combien de temps il faut au sol pour revenir à une concentration de Cu et de Zn disponible équivalant à celle relevée avant les amendements, une fois ceux-ci terminés. Les taux d'application étaient les suivants : 0, 30, 60 ou 90 Mg de fumier par hectare pour les parcelles soumises à un régime pluvial et 0, 60, 120 ou 180 Mg de fumier par hectare (poids humide) pour les parcelles irriguées. Bien qu'on ait épandu du fumier pendant 45 ans sur certaines parcelles, les applications ont été interrompues au bout de 14 ans pour un sous-groupe d'entre elles et après 30 ans pour un deuxième sous-ensemble, de manière à permettre l'étude des effets résiduels après 31 et 15 années d'application, respectivement. Des échantillons de sol ont été prélevés à l'automne 2003, 2008, 2013 et 2018, puis analysés pour déterminer la concentration de Cu et de Zn disponible. Les parcelles ont été cultivées continuellement et la concentration de Cu et de Zn a été mesurée dans l'ensilage et le grain après la récolte. D'après le modèle de régression élaboré grâce aux données recueillies, les effets résiduels persistent longtemps et il faut compter respectivement 10 à 20 ans et 23 à 41 ans pour que le sol des parcelles irriguées revienne à une concentration de Cu et de Zn identique à celle qui précédait les amendements, contre 10 à 24 ans pour le Cu et 21 à 32 ans pour le Zn, dans le sol des parcelles soumises au régime pluvial. L'application prolongée de fumier de bovins pourrait aboutir à une accumulation de Cu et de Zn, ce qui aurait de longs effets résiduels sur le sol, avec le risque accru qu'on voie ces éléments s'infiltrer dans la nappe phréatique. [Traduit par la Rédaction]

*Mots-clés :* fumier de bovins, charge de Cu et de Zn, effets résiduels, expériences sur le terrain de longue haleine, taux d'application du fumier.

Received 25 September 2020. Accepted 20 December 2020.

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## Introduction

Applying organic manure benefits soil in many ways, including improving its quality through carbon sequestration and productivity by enhancing micro- and macronutrients in soils (Poblete-Grant et al. 2020; Hill et al. 2021). However, excessive accumulation of some nutrients can arise from the long-term use of manures, relative to the use of fertilizers because the ratio of nutrients in manures is different from the ratio of nutrients removed by common crops (Edmeades 2003; Diacono and Montemurro 2011). Accumulation and legacy effects have also been reported for nitrogen (N) and phosphorus (P) (Indraratne et al. 2009) and soil microbial community composition (Zhang et al. 2018) due to long-term manure applications. Soil potassium (K), calcium (Ca), iron (Fe), zinc (Zn), and copper (Cu) concentration increased significantly in cattle and swine manure after 10 yr of application compared with their initial levels, suggesting that they are good sources of many essential nutrients, in places where nutrients are limiting but can lead to excessive accumulation and increased environmental risk (Schlegel et al. 2017).

Cattle raised in Canadian feedlots are often fed diets supplemented with at least 17 minerals, including Cu and Zn (National Research Council 2000). Thus, applying their manure to nearby soils has become a growing source of metal input. The same effect is occurring due to nutritional supplementation across intensive animal production industries (Bolan et al. 2004); repeated applications of poultry litter (Schomberg et al. 2008), pig manure (Novak et al. 2004; Xu et al. 2013), and cattle manure (Lipoth and Schoenau 2007; Benke et al. 2008) have all led to significant increases in Cu and Zn contents in the upper soil layer. Livestock manure sources contributed 69% and 51% of total Cu and Zn inputs, respectively, in arable lands of China (Luo et al. 2009). Significant increase of total concentrations of soil Cd, Zn, Cr, and Cu observed after 15 yr of continuous manure application at  $\sim 100 \text{ Mg}\cdot\text{ha}^{-1}$  in a protected-field vegetable production system (Zhen et al. 2020). Application of swine manure at a rate of  $\sim 250 \text{ Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  for 4 yr created a very high environmental risk of leaching due to soil Cu and Zn accumulation (Qian et al. 2018). Increased Cu and Zn concentrations in soil samples were found with the application of wastes derived from animal farming (Mantovi et al. 2003; Qian et al. 2018).

Most crops use only a small amount of Cu and Zn to complete their life cycles, and excess beyond plant uptake can lead to accumulation in the soil. The critical concentration of Cu and Zn in agronomic crops varies widely (Whitehead 2000). Tucker et al. (2003) reported Mehlich 3 extractable critical concentrations in North Carolina soils that can be phytotoxic to sensitive plants as  $>60 \text{ mg}\cdot\text{kg}^{-1}$  for Cu and  $>120 \text{ mg}\cdot\text{kg}^{-1}$  for Zn. Thus, Cu and Zn contents in agricultural soils could increase to levels exceeding plant requirements, and even to

levels toxic to the plants (McBride and Spiers 2001). High Cu and Zn loadings due to long-term manure applications were subjected to leaching resulting topsoil Cu and Zn concentrations below the level considered to be phytotoxic, even after the total Cu and Zn in soils increased by 204% and 107%, respectively (Xu et al. 2013). Copper enrichment in subsoil was evident after 10 yr of swine manure applications even though Cu concentrations in topsoil were far below concentrations considered phytotoxic to sensitive crops (Novak et al. 2004). Zinc migrated to a depth of 30 cm in a field with a 40 yr history of poultry manure application, whereas in other fields ( $<40$  yr of history), Cu and Zn had accumulated within the 0–17.5 cm depth (Brock et al. 2006). Hence, accumulation of Cu and Zn in topsoil due to manure applications could lead to phytotoxicity in plants or leaching raising environmental concerns of groundwater contamination. However, Sukkariyah et al. (2005) reported no adverse effects on plant uptake or growth after 17–19 yr of single biosolid application at a rate of  $210 \text{ Mg}\cdot\text{ha}^{-1}$ , although available Cu and Zn became significantly greater than in the control site. Annual addition of animal manures at rates of approximately  $100 \text{ kg N}\cdot\text{ha}^{-1}$  for 3–5 yr did not constitute an environmental risk from Cu and Zn loading in soils (Qian et al. 2003).

Long-term field experiments, i.e., lasting decades and longer, examining continuous manure applications provide data on changes in the soil's heavy metal content and, along with measurements of residual effects after years of discontinuation, are useful for predicting the fate of metals in soil–plant–environment interactions. However, such research on the effects of metal accumulation in soils following addition of composted materials is very limited (Diacono and Montemurro 2011). Studying the environmental fate of heavy metals, such as Cu and Zn, in a range of compost-amended soils is very important because metal behaviours depend on soil characteristics, climatic conditions, and microbial activities (Businelli et al. 2009). A few studies have examined on the legacy effects and recovery times for metals contained in manure applications at recommended rates (Brock et al. 2006) or in fertilizer applications (Xiaorong et al. 2007); a one-time biosolid application at higher rates than recommended has also been studied (Sukkarivah et al. 2005). However, heavier applications of manure to soils are known to occur as a result of increasing livestock operations (Liu et al. 2020). No investigations could be found on the status of Cu and Zn in soil and their legacy effects following repeated applications of manure for over 40 or more years. The objectives of our study were to (i) investigate Cu and Zn accumulation in surface and subsoil layers following 14–45 yr of cattle feedlot manure applications at different rates under rainfed and irrigated crop cultivation systems and (ii) estimate time period necessary to recover from the residual effects of available Cu and Zn in soils after

**Table 1.** Manure application rates, number of years of continuous application, and number of years after discontinuation of manure in irrigated and rainfed treatments.

Treatment <sup>a</sup>	2003		2008		2013		2018	
	Cont. <sup>b</sup>	Discont. <sup>c</sup>	Cont.	Discont.	Cont.	Discont.	Cont.	Discont.
Mi0/Mr0	0	0	0	0	0	0	0	0
Mif/Mrf	13	0	18	0	23	0	28	0
Mi/Mr	30	0	35	0	40	0	45	0
DDi/DDr	30	0	30	5	30	10	30	15
Di/Dr	14	16	14	21	14	26	14	31

<sup>a</sup>M, manure continuous applications since 1973; DD, delayed discontinued manure in 2003; D, discontinued manure in 1987; i = irrigated, r = rainfed; 0 = control; f = fertilizer N added beginning in 1990.

<sup>b</sup>Number of years of continuous manure/fertilizer application.

<sup>c</sup>Number of years after discontinuation of manure application (legacy years).

discontinuation of manure applications for 15–31 yr. We hypothesize that the longer and larger the rate of cattle feedlot manure applications, the greater the Cu and Zn concentrations in soils under both rainfed and irrigated soils. This study gave us an opportunity to study the impact of long-term manure applications on soil Cu and Zn concentrations and, subsequently, the legacy effect (following discontinuation of manure applications for 15–31 yr) of Cu and Zn in soils.

## Materials and Methods

### Experimental design

The experiment was conducted on a Dark Brown Chernozemic clay loam soil (Typic Haploboroll) at the Agricultural and Agri-Food Canada Research Centre in Lethbridge, AB. This region is semiarid, with an average temperature of 14.7 °C and 251 mm rainfall from May to September (Benke et al. 2013). Details on the split-split-plot design and soil properties were provided by Sommerfeldt and Chang (1985). Briefly, starting in fall 1973, cattle feedlot manure was applied annually to nine replications at 0, 30, 60, and 90 Mg·ha<sup>-1</sup>·yr<sup>-1</sup> (wet weight) to the rainfed block (manure treatments Mr0, Mr30, Mr60, and Mr90) and at 0, 60, 120, and 180 Mg·ha<sup>-1</sup>·yr<sup>-1</sup> to the irrigated block (manure treatments Mi0, Mi60, Mi120, and Mi180). In 1987, following 14 yr of continuous annual manure applications, three replications under rainfed (discontinued treatments Dr30, Dr60, and Dr90) and two replications under irrigated conditions (discontinued treatments Di60, Di120, and Di180) received no further manure application to investigate the residual effect of 14 annual cattle manure applications. In 2003, following 30 yr of continuous annual manure applications, three replications under rainfed (delayed discontinued treatments DDr30, DDr60, and DDr90) and two replications under irrigated conditions (delayed discontinued treatments DDi60, DDi120, and DDi180) received no further manure application to investigate the residual effect of 30 annual cattle manure applications. Three replications under both rainfed and irrigated conditions continued to receive annual manure

applications, reaching a total of 45 yr in 2018 (Table 1). From 1973 to 1989, the experimental design was a split plot, with tillage treatments as main plots and manure treatments as subplots. The tillage treatments included three methods (plow, rototill, and cultivator plus disk) of incorporating manure into the soil, each randomly assigned and replicated three times. Since 1990, manure in all plots has been incorporated with a cultivator.

Control treatments (Mi0/Mr0) never received any amendment application. Fertilized rainfed and irrigated treatments (Mrf/Mif) received ammonium nitrate annually at rates of 50 and 100 N·ha<sup>-1</sup> respectively, beginning in 1990. Barley was the main crop grown in most years, except 1996 (canola), 1997 (corn in irrigated), 1998 (triticale in rainfed), 1999 and 2000 (triticale), 2016 (soybean) and 2018 (soybean). Irrigated treatments received an average of 160 mm water·yr<sup>-1</sup>.

### Sample preparation and analysis

The manure was obtained from an open, unpaved commercial feedlot near Coaldale, AB, over the 45 yr of application. Manure Cu and Zn loadings are given in Table 2 according to the composition of the feedlot manure. Manure samples collected during annual applications were air-dried and coarsely ground (<2 mm), then a finely ground (<0.15 mm) subsample was used for Cu and Zn analysis. The Cu and Zn concentrations in manure samples were analyzed using 0.25 g finely ground samples weighed into 50 mL plastic digestion vials with 5 mL of 50% HNO<sub>3</sub> and 2 mL of 30% H<sub>2</sub>O<sub>2</sub> added. Each plastic vial containing a sample was digested for 1 h at 90 °C with another 2 mL of 30% H<sub>2</sub>O<sub>2</sub> added halfway during the process. Samples were brought to 50 mL with ultra-pure water and passed through a 0.45 µm Teflon filter. The Cu and Zn were measured using an atomic absorption spectrometer (Varian Model AA240, Varian Inc., Palo Alto, CA, USA).

Archived soil samples collected in fall 2003, 2008, 2013, and 2018 after crop harvest and before annual manure application from two depths, 0–15 and 15–30 cm, were analyzed for available Cu and Zn (McKeague et al. 1978) to measure the plant-available pool of metals

**Table 2.** Estimated manure, copper (Cu) and zinc (Zn) loadings to the soils and Cu and Zn removal from soils as crop uptake by 2018 by different treatments.

Treatment <sup>a</sup>	Loading			Removal <sup>b</sup>		% Removal	
	Manure (Mg·ha <sup>-1</sup> )	Cu (kg·ha <sup>-1</sup> )	Zn (kg·ha <sup>-1</sup> )	Cu (kg·ha <sup>-1</sup> )	Zn (kg·ha <sup>-1</sup> )	Cu	Zn
Mr0	0	0	0	3.92 (±1.5)	10.86 (±1.5)	NA	NA
Mrf	0	0	0	4.15 (±1.8)	10.28 (±1.8)	NA	NA
Mr30	392	18	124	3.63 (±1.6)	12.45 (±1.6)	20.1	10
Mr60	783	37	249	4.96 (±1.4)	15.36 (±0.9)	13.4	6.2
Mr90	1175	55	373	3.23 (±0.2)	12.64 (±1.4)	5.8	3.4
DDr30	261	12	83	4.01 (±0.5)	13.53 (±1.0)	33.5	16.3
DDr60	522	25	166	5.11 (±2.1)	13.92 (±3.7)	20.4	8.4
DDr90	783	37	249	3.30 (±0.3)	15.98 (±2.7)	8.9	6.4
Dr30	122	6	39	3.71 (±0.7)	9.35 (±2.1)	61.8	23.9
Dr60	244	11	77	5.37 (±2.5)	10.37 (±4.2)	48.8	13.5
Dr90	365	17	116	4.35 (±0.9)	8.89 (±1.3)	25.6	7.6
Mi0	0	0	0	6.34 (±1.8)	16.26 (±2.7)	NA	NA
Mif	0	0	0	5.77 (±0.9)	20.46 (±2.3)	NA	NA
Mi60	783	37	249	6.61 (±1.3)	21.29 (±1.4)	17.8	8.5
Mi120	1566	74	498	11.08 (±4.1)	30.86 (±7.8)	14.9	6.2
Mi180	2349	110	747	4.77 (±0.5)	26.13 (±1.0)	4.3	3.4
DDi60	522	25	166	5.24 (±0.5)	25.10 (±7.3)	20.9	15.1
DDi120	1044	49	332	5.71 (±0.7)	26.80 (±2.7)	11.6	8.1
DDi180	1566	74	498	6.09 (±0.3)	26.51 (±4.2)	8.2	5.3
Di60	244	11	77	5.62 (±1.3)	23.45 (±4.6)	51.1	30.4
Di120	487	23	155	5.21 (±1.6)	19.46 (±3.3)	22.7	12.5
Di180	731	34	232	6.67 (±0.2)	22.02 (±0.7)	19.6	9.4

**Note:** NA, not applicable.

<sup>a</sup>M, manure continuous applications since 1973; DD, delayed discontinued manure in 2003; D, discontinued manure in 1987; i = irrigated, r = rainfed; 0 = control; f = fertilizer N added beginning in 1990; 30, 60, 90, 120, and 180 = manure rates in Mg·ha<sup>-1</sup>.

<sup>b</sup>Standard deviation is given in the parentheses.

in the soil. In brief, 12.5 g of soil were shaken for 2 h in 25.0 mL of diethylenetriaminepentaacetic acid + triethanolamine (DTPA-TEA) extractant and filtered using Whatman No. 42 filter paper. Copper and Zn analyses were carried out on a Thermo Scientific iCAP6300 Duo Inductively Coupled Plasma Atomic Emission (ICP-AES) Spectrometer. Soil pH was measured in 1:2 soil-to-water ratio using an Accumet AB pH meter (Fisher Scientific, Hampton, NH, USA).

Total yield records of 2003, 2008, 2013, 2017, and 2018 were used for this study. Half of the crops of each subplot were harvested at the soft dough stage as forage feed, and the remaining half were harvested at maturity as grains. Silage and grain samples of barley (2003, 2008, 2013, and 2017) and soybean (2018) were analyzed for total Cu and Zn as described above for manure analysis and were expressed in dry-matter basis.

#### Data handling and statistical analysis

Cumulative manure applied (dry weight) for each treatment was estimated by multiplying the average annual manure applied (dry weight) by the number of years of manure application. Total Zn and Cu loadings were also estimated similarly by multiplying average

Zn or Cu concentrations in manure by the number of years of manure applied (Table 2).

Barley samples from 2003, 2008, 2013, and 2017 were used to estimate cumulative Cu and Zn removals as harvest during the 45 yr of cultivation. Average yield, Cu and Zn values of barley in 2003 corrected for 30 yr, and average values of barley in 2008, 2013, and 2017 corrected for 5 yr, each were summed to estimate cumulative Cu and Zn removals as harvests from the soils of each treatment (Table 2) at the end of 45 yr. Total yield removed from the fields were calculated similarly using the average yields and number of years of cultivation.

Separate analyses were performed for rainfed and irrigated treatments using the MIXED procedure in SAS version 6.09 (SAS Institute Inc. 2005) for the analysis of variance with manure rate and interaction in the model as fixed effects, and the replication by manure interaction as a random effect. The UNIVARIATE procedure was used to check the residuals for normality and for potential outliers. Means were separated by the least significant difference test and were considered significant at  $P < 0.05$ . Pearson's correlation analysis was employed to identify the relationship between manure loads with Cu or Zn in soils. Regressions were made between Cu

**Table 3.** Correlations (*r*) between copper (Cu) or zinc (Zn) contents at 0–15 and 15–30 cm depths under irrigated and rainfed conditions with manure loadings and number of years of continuous manure applications (0–45 yr).

Parameter	Cu (mg·kg <sup>-1</sup> )				Zn (mg·kg <sup>-1</sup> )			
	Irrigated		Rainfed		Irrigated		Rainfed	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Manure loading (kg·ha <sup>-1</sup> )	0.80***	0.59***	0.76***	0.10	0.84***	0.78***	0.85***	0.78***
Years of manure application	0.73***	0.47**	0.71***	0.15	0.69***	0.62***	0.71***	0.69***

Note: \*\*\*, \*\*, and \* represent significant relationships at 0.001, 0.01, and 0.05, respectively.

and Zn in soils and number of years of discontinuation (legacy effect) to understand the removal rate of Cu and Zn and to estimate the number of years following discontinuation necessary to reduce Cu or Zn levels to control values.

## Results and Discussion

### Manure, Cu and Zn loadings in soil, and removal of Cu and Zn in crop yields

On average, the manure in this study contained 195.6 ± 12.1 g C·kg<sup>-1</sup>, 17.0 ± 0.2 g N·kg<sup>-1</sup>, 6.6 ± 0.1 g P·kg<sup>-1</sup>, 47 ± 1.1 mg Cu·kg<sup>-1</sup>, and 318 ± 14.4 mg Zn·kg<sup>-1</sup> (data not shown). Forty-five continuous annual manure applications at the 180 Mg·ha<sup>-1</sup>·yr<sup>-1</sup> rate added the most manure (2349 Mg·ha<sup>-1</sup>), Cu (110 kg·ha<sup>-1</sup>), and Zn (747 kg·ha<sup>-1</sup>) loadings to soils (Table 2) among irrigated treatments. The corresponding rainfed treatment with the highest manure rate (Mr90) received 1175 Mg·ha<sup>-1</sup> manure including 55 kg Cu·ha<sup>-1</sup> and 374 kg Zn·ha<sup>-1</sup>. Delayed discontinued irrigated treatments (DDi) received a maximum of 74 kg Cu·ha<sup>-1</sup> and 498 kg Zn·ha<sup>-1</sup> at the 180 Mg·ha<sup>-1</sup>·yr<sup>-1</sup> rate over 30 yr of manure applications, whereas the maximum for rainfed treatments (DDr90) was 37 kg Cu·ha<sup>-1</sup> and 249 kg Zn·ha<sup>-1</sup>. Treatments discontinued in 1987 (Di/Dr) received 34 Cu kg·ha<sup>-1</sup> and 232 Zn kg·ha<sup>-1</sup> in irrigated and 17 kg Cu·ha<sup>-1</sup> and 116 kg Zn·ha<sup>-1</sup> in rainfed treatments, at the highest manure rate over 14 yr of manure applications. Generally, because of the manure composition, each application added almost seven times as much Zn as Cu to soils.

Crop harvest removed total of 3.2–5.3 and 4.7–11.1 kg Cu·ha<sup>-1</sup> and 8.9–15.9 and 16.2–30.8 kg Zn·ha<sup>-1</sup> from rainfed and irrigated treatments, respectively (Table 2). Total Cu removals from soils accounted 6%–62% in rainfed and 4%–51% in irrigated treatments added from manure applications. Similarly, total Zn removals from soils accounted 3%–23% in rainfed and 3%–30% in irrigated treatments. Crop removal played more significant role on Cu accumulations in soils than for Zn.

### Manure loadings on pH, DTPA-extractable Cu and Zn in soil

Soil pH fluctuated within a narrow range of 7.14–7.95 in rainfed and 7.03–7.90 in irrigated treatments at

0–15 cm over the years of 2003–2018 (data not shown). Soil pH was negatively correlated with manure loads at 0–15 cm depth for rainfed ( $r = -0.6$ ;  $p = 0.001$ ) and irrigated ( $r = -0.38$ ;  $p = 0.05$ ) treatments. Addition of cattle feedlot manure, which had a 45 yr average pH of 7.0 ± 0.03, reduced soil pH in both rainfed and irrigated treatments.

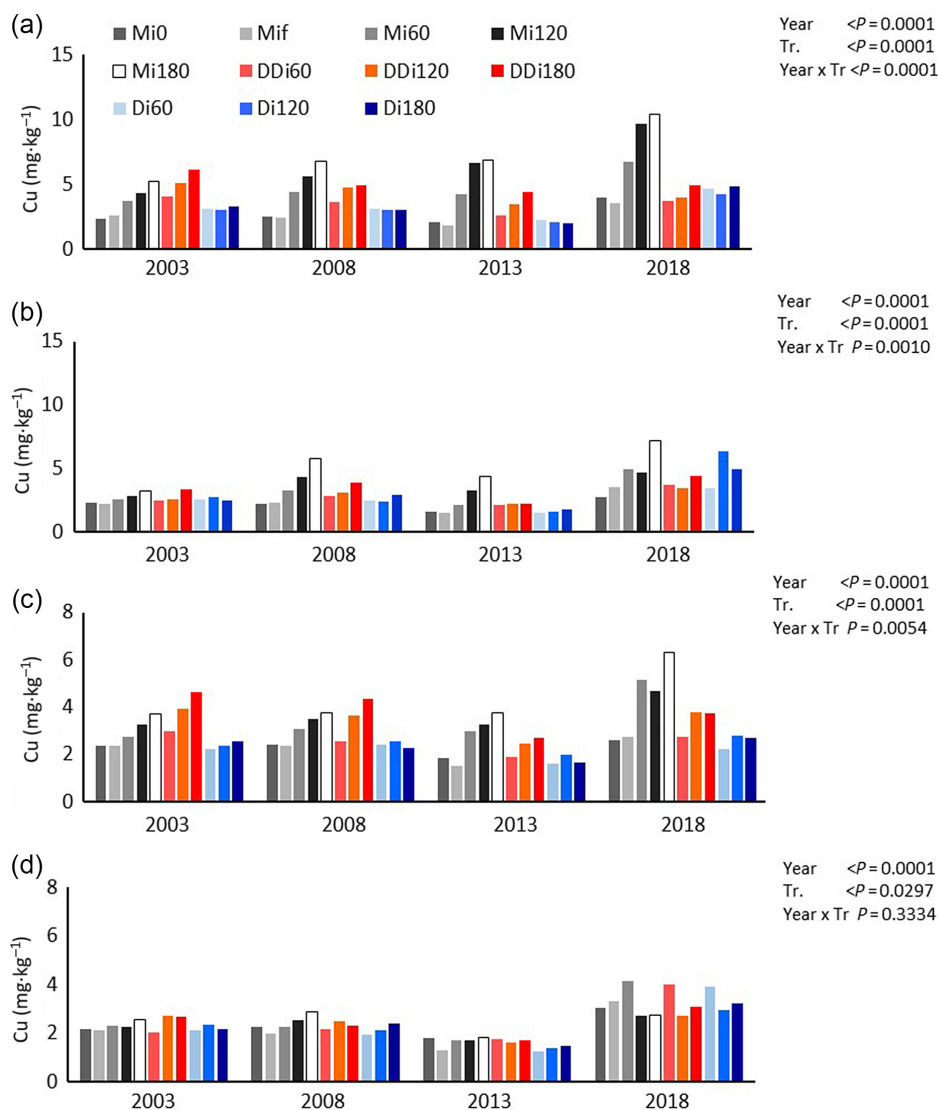
Copper concentrations in soil significantly increased with increasing manure loadings in irrigated treatments at both 0–15 ( $r = 0.80$ ,  $p = 0.001$ ) and 15–30 cm ( $r = 0.59$ ,  $p = 0.001$ ) depths and rainfed treatments at 0–15 cm ( $r = 0.76$ ,  $p = 0.001$ ) depth (Table 3). The Cu in the manure increased DTPA-extractable Cu in soils in both 0–15 and 15–30 cm depths under irrigated conditions. Under rainfed conditions, manure also increased DTPA-extractable Cu in the 0–15 cm depth but not in the 15–30 cm depth; this can be attributed to the drier conditions that limited Cu mobility leaving most in the surface layer. A significant correlation was found between number of years of manure applications and DTPA-extractable Cu in soils in irrigated and rainfed treatments. The longer the years of manure applications, the greater the Cu contents in soils in both 0–15 ( $r = 0.73$ ,  $p = 0.001$ ) and 15–30 cm ( $r = 0.47$ ,  $p = 0.01$ ) depths of irrigated and the 0–15 cm ( $r = 0.71$ ,  $p = 0.001$ ) depth of rainfed treatments.

Correlations between manure loadings and DTPA-extractable Zn in soils confirmed significant relationships in both 0–15 ( $r = 0.84$ ,  $p = 0.001$ ) and 15–30 cm ( $r = 0.85$ ,  $p = 0.001$ ) depths of irrigated and rainfed ( $r = 0.78$ ,  $p = 0.001$  and  $r = 0.78$ ,  $p = 0.001$ , for 0–15 and 15–30 cm, respectively) treatments (Table 3). Manure applications over 45 yr were significantly responsible for Zn enrichments in both rainfed and irrigated soils at both depths, as explained with Zn loadings. In a study introducing organic Zn complexes to agricultural soils, Zn migrated in soil profiles faster than the control leading to Zn leaching (Alvarez et al. 2001). Hence, formation of organo-Zn chelates can be expected in these manure treatments enhancing Zn mobility and migration to subsurface layers.

### Continuous manure application and residual effects on Cu concentrations in soils

Effects of manure rate (treatment) and year of sampling and treatment × year interaction effects were

**Fig. 1.** Copper (Cu) extracted from soils over the years of manure applications at different rates under irrigated ((a) 0–15 cm and (b) 15–30 cm depths) and rainfed ((c) 0–15 cm and (d) 15–30 cm depths) conditions.



significant for DTPA-extractable Cu in irrigated (both 0–15 and 15–30 cm depths) and rainfed (0–15 cm depth) treatments (Fig. 1). The control treatment (Mi0) showed the lowest ( $2.36 \text{ mg}\cdot\text{kg}^{-1}$ ) Cu in soil as expected. The fertilizer treatment (Mif) which received only N fertilizers, behaved similarly to Mi0, with respect to Cu concentration. The highest Cu in 2003 in the 0–15 cm depth was  $6.16 \text{ mg}\cdot\text{kg}^{-1}$  for the longest continuous treatment (30 yr) at the highest manure rate ( $180 \text{ Mg}\cdot\text{ha}^{-1}$ ) under irrigation (Fig. 1a). Copper concentrations in the 0–15 cm depth under irrigation were ordered as follows: (2003) Mi180, DDi180 > Mi120, DDi120 > Mi60, DDi60 > all Di treatments and Mi0, Mif; (2008) Mi180 > Mi120, DDi180 > DDi120, Mi60 > DDi60, all Di treatments, Mif, Mi0; (2013) Mi180, Mi120 > DDi180, Mi60, DDi120 > DDi60, all Di, Mi0, Mif; (2018) Mi180, Mi120 > Mi60 > all DDi, all Di, Mi0, Mif. The highest Cu in the 0–15 cm depth was

reported for Mi180 across all sampling years. All treatments discontinued in 1987 (Di) showed Cu concentrations similar to the controls from 2003 to 2018, indicating Cu loadings added to soils for 14 consecutive years (from 1973 to 1987) were removed from the 0–15 cm depth within 16 yr after discontinuation. The Cu in the 0–15 cm depth for DDi treatments, for which manure applications were terminated in 2003, similarly declined to control Cu levels within 15 yr (by 2018).

Forty-five years of long-term manure applications at rates of 60, 120, and  $180 \text{ Mg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  accumulated DTPA-extractable Cu in soils from 6.7 to  $10 \text{ mg}\cdot\text{kg}^{-1}$  in the 0–15 cm depth. After discontinuing manure applications for 15 yr in DDi treatments, Cu levels were significantly lower in the 0–15 cm depth than continuous treatments, and levels in 2018 were comparable to controls (Mi0 and Mif). This further confirms the 2003

results indicating 15 yr without manure applications are necessary to eliminate the Cu legacy effect in the 0–15 cm depth in irrigated treatments. A similar trend was observed with Cu at 15–30 cm but at a lower concentration than the 0–15 cm depth. The highest Cu contents were observed with the Mi180 treatment, and the lowest were in Mi0 and Mif treatments in all years in the 15–30 cm depth (Fig. 1b). It is worth mentioning that the Mi180 treatment increased DTPA-extractable Cu in the 15–30 cm depth to  $7.2 \text{ mg}\cdot\text{kg}^{-1}$  in 2018. We can expect relatively more Cu loss to leaching at 15–30 cm than at the 0–15 cm depth if the root system does not reach that depth. Formation of stable complexes with soil components, leaching and plant uptake are possible reasons for the lower Cu concentrations in the 15–30 cm depth. A previous study found leaching accounted for 40% of total Cu losses and forming complexes with minerals and organic matter accounted for 76%–92% respectively, of the total Cu remaining in soil after 17 yr of fertilizer application (Xiaorong et al. 2007). The increase in soil organic matter will decrease Cu availability as Cu strongly associates with soil organic matter binding as an inner-sphere complex (Han et al. 2001). A comparison of various isotherms and metal competitions in various soils indicate that Cu is adsorbed more strongly than Zn with soil components (Arias et al. 2006; Elbana et al. 2018).

Similar trends as for irrigated treatments were observed in rainfed treatments (Figs. 1c and 1d) for Cu; the highest Cu concentrations followed the longest application times and highest rates. Copper content was reduced after manure treatments were discontinued, and Cu was higher in the 0–15 cm than 15–30 cm depth. A soil metal study conducted after 25 annual manure applications (Benke et al. 2008) reported significant increases in total Zn and Cu content in irrigated manure treatments.

Available Cu concentrations in soils in 2018 were greater than the values of 2003, 2008, or 2013 in all treatments, including controls (Fig. 1). Crop data showed that due to crop failure, reduced Cu removals in 2018 led to Cu accumulations in soils (Supplementary Table S1<sup>1</sup>). Copper removals by crops in 2018 were 2%–85%, 89%–96%, and 40%–86% less than the amounts removed in 2003, 2008, and 2013, respectively. Further as explained previously, 2018 had the lowest soil pH facilitating Cu solubility in soils. Less Cu removal from soils through yields and lower pH may have contributed to higher-than-expected Cu levels in soils in 2018.

#### Continuous manure application and residual effects on Zn concentrations in soils

Manure rate (treatment), year of sampling, and treatment  $\times$  year interaction effects were significant for Zn

contents in both irrigated and rainfed treatments at both 0–15 and 15–30 cm depths (Fig. 2). Increased Zn in soil with higher manure rates were observed in both rainfed and irrigated treatments at both 0–15 and 15–30 cm depths. By looking at the statistical differences in Zn contents in soils among treatments, we can see following trends in the irrigated 0–15 cm depth: (2003) Mi180, DDi180 > Mi120, DDi120 > Mi60, DDi60 > Di180 > Di120, Di60 > Mif, Mi0; (2008) Mi180, Mi120, DDi180 > DDi120, Mi60, DDi60 > Di180 > Di120, Di60 > Mi0, Mif; (2013) Mi180 > Mi120 > DDi180 > Mi60 > Di180, Di120, Di60 > Mi0, Mif; and (2018) Mi180 > Mi120 > Mi60 > DDi180 > DDi120, DDi60 > Di180, Di120 > Di60, Mi0, Mif. In brief, Zn contents in irrigated 0–15 (Fig. 2a) and 15–30 cm (Fig. 2b) and rainfed 0–15 (Fig. 2c) and 15–30 cm (Fig. 2d) varied from 8 to 107, 5 to 62, 5 to 45, and 1 to 11  $\text{mg}\cdot\text{kg}^{-1}$ , respectively, among treatments. At the recommended manure rate, Mi60, there was 40  $\text{mg}\cdot\text{kg}^{-1}$  of DTPA-extractable Zn in 2018, after 45 yr of repeated manure applications. According to the previous studies, critical total Zn in soils established as 70–400  $\text{mg}\cdot\text{kg}^{-1}$  (Mantovi et al. 2003), DTPA-extractable Zn for maize and wheat crops as 7 and 11  $\text{mg}\cdot\text{kg}^{-1}$ , respectively (Takkar and Mann 1978) and Mehlich 3-extractable Zn to become phytotoxic to sensitive plants as >120  $\text{mg}\cdot\text{kg}^{-1}$  (Tucker et al. 2003). Manure applications even at recommended rates could lead to toxic Zn levels for some crops when applied for a long period of time. Application of animal manure to meet the N requirements or P removal rates of agronomic crops can lead to accumulation of micronutrients in soil. There are differences among micronutrients in their likelihood to become enriched in soils; addition of manure significantly enriched Zn, Fe, and Mn in soils more than the Cu (Wei et al. 2006).

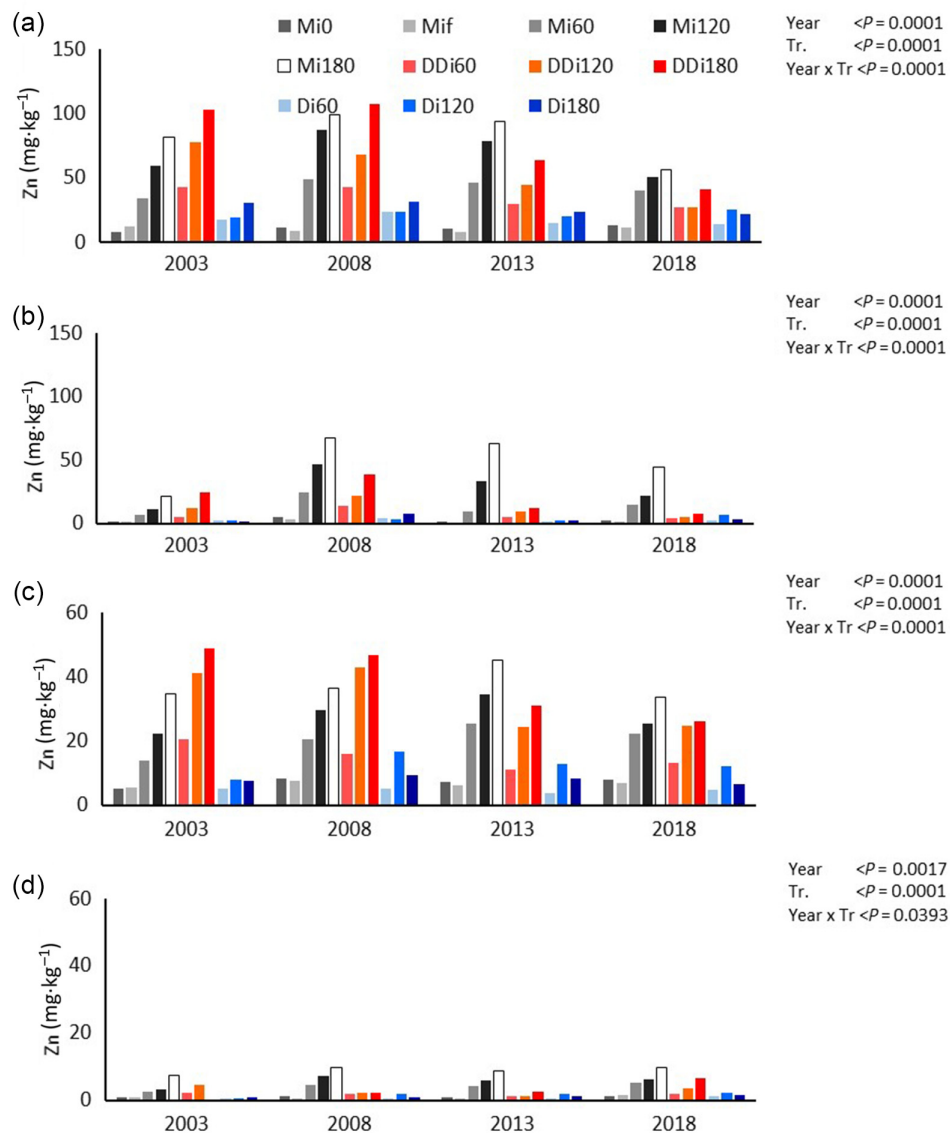
Treatment Mi120, at double the recommended rate, showed Zn levels between 51 and 81  $\text{mg}\cdot\text{kg}^{-1}$  during the years of analysis, the lowest being reported in 2018. Similar observations were made for Mi180; Zn ranging from 56 to 107  $\text{mg}\cdot\text{kg}^{-1}$  and the lowest in 2018. Low DTPA-extractable Zn in 2018 could be due to plant uptake, migration to lower depths of soil, complexation with organic matter, change of pH or formation of any secondary precipitations. According to previous research, Zn behaves differently with soil organic matter; the solid form of organic matter decreases Zn solubility by sorbing Zn on to surface functional groups (Boguta and Sokolowska 2016), whereas the complexation of Zn with dissolved organic compounds increases Zn solubility and mobility (Houben and Sonnet 2012).

The legacy effect of Zn was more pronounced than Cu following manure application. Following 14 yr of manure applications at two or three times higher than recommended levels (Di120 and Di180), Zn levels in soils remained higher than in the control treatment (Mi0)

<sup>1</sup>Supplementary data are available with the article at <https://doi.org/10.1139/cjss-2020-0124>.



**Fig. 2.** Zinc (Zn) extracted from soils over the years of manure applications at different rates under irrigated ((a) 0–15 cm and (b) 15–30 cm depths) and rainfed ((c) 0–15 cm and (d) 15–30 cm depths) conditions.



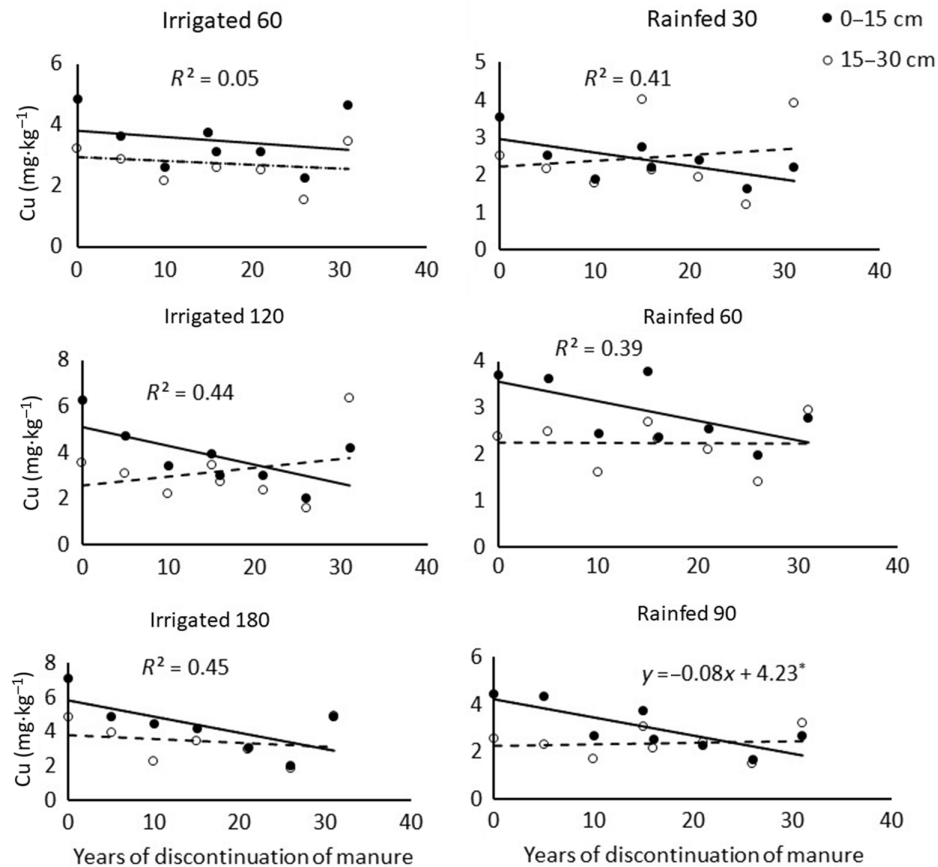
even 31 yr after manuring had been discontinued, indicating the legacy effect. Using the recommended manure rate for irrigated fields, i.e., 60 Mg·ha<sup>-1</sup>·yr<sup>-1</sup>, for 14 yr, followed by 21 yr of discontinuation Zn levels returned to the control (Mi0) level in 2008. In general, significantly higher Zn was found in the continuous treatments (Mi60, Mi120, and Mi180) than their corresponding discontinued treatments after 14 yr (Di60, Di120, and Di180) or 30 yr of manure applications (DDi60, DDi120, and DDi180), followed by 5–31 yr of discontinuation.

#### Cu and Zn legacy effects and time to recover

The experimental design consisted of long-term continuous manure applications, 14–30 yr, followed by discontinuation for 15–31 yr. Regressions were determined between Cu in soils with the number of years after

discontinuing manure applications for irrigated and rainfed treatments at 0–15 and 15–30 cm depths to calculate time to reach M0 level (Fig. 3). Available Cu levels fluctuate in a narrow range within each irrigated treatment; for example, Mi60 from 2.2 to 4.8, Mi120 from 2.1 to 6.2, Mi180 from 1.8 to 7.1. Average Cu concentration for the irrigated control (Mi0) in the 0–15 cm depth was 2.76 mg·kg<sup>-1</sup> and for 15–30 cm was 2.31 mg·kg<sup>-1</sup>. Because Cu fluctuates within very narrow range and Cu returned to control concentrations during the study period, the regression between number of years of manure discontinuation and Cu in soil was not significant for irrigated manure rates. From Fig. 3, Mi60 and Mi120 returned to the Mi0 level in about 10 yr, whereas Mi180 required 15–20 yr. A similar pattern of Cu concentrations in soils with years of manure discontinuation could be observed with rainfed treatments, although

**Fig. 3.** Relationships between years after discontinuation of manure application with copper (Cu) in soil under irrigated and rainfed treatments at 0–15 and 15–30 cm depths (\* represent significant relationship at 0.05 probability;  $R^2$  values for 0–15 cm depth are shown; equations are given only for significant relationships; control (Mi0) Cu levels for irrigated 0–15 and 15–30 cm depths were 2.76 and 2.31 mg·kg<sup>-1</sup>, respectively, and for rainfed (Mr0) for both depths was 2.30 mg·kg<sup>-1</sup>).

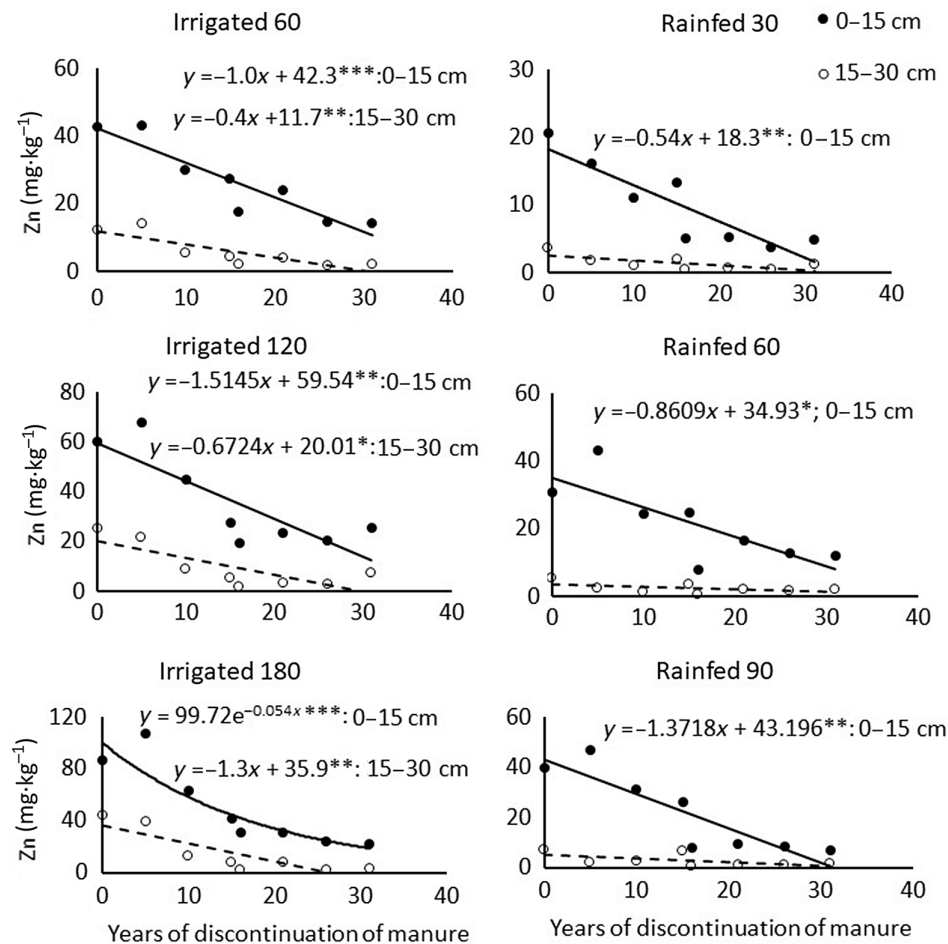


Mr90 had a significant relationship ( $R^2 = 0.63$ ,  $p = 0.05$ ) in the 0–15 cm depth (Fig. 3). The time to return to Mr0 Cu concentrations (2.31 mg·kg<sup>-1</sup>) in rainfed treatments was estimated for Mr30 as about 10 and for Mr60 as 15–20 yr. The time required for Mr90 to reach Mr0 Cu levels can be calculated as 24 yr using the regression equation. Because Cu returned to control levels during the experimental time for DD and D treatments, we can conclude that the Cu legacy effect disappeared 10–20 yr and 10–24 yr after discontinuation of manure applications for irrigated and rainfed, respectively, depending on the rate of manure applied.

Regressions were calculated between Zn in soils and the number of years following discontinuation of manure applications for irrigated and rainfed treatments at 0–15 and 15–30 cm depths (Fig. 4) to investigate the legacy effect of Zn. Following discontinuation of manure applications, Zn concentrations declined continuously over the next 31 yr. However, unlike Cu, even after 31 yr Zn concentrations remained high (>20 mg·kg<sup>-1</sup>) in the 0–15 cm depth of some irrigated treatments. The times required for Zn to decline to its corresponding control values were calculated using

regression equations in Fig. 4. Under irrigation, significant linear relationships were observed at both depths of 60 Mg·ha<sup>-1</sup> ( $R^2 = 0.86$ ,  $p = 0.001$  for 0–15 cm and  $R^2 = 0.72$ ,  $p = 0.01$  for 15–30 cm), 120 Mg·ha<sup>-1</sup> ( $R^2 = 0.69$ ,  $p = 0.01$  for 0–15 cm and  $R^2 = 0.62$ ,  $p = 0.05$  for 15–30 cm) and 15–30 cm depth of 180 Mg·ha<sup>-1</sup> ( $R^2 = 0.71$ ,  $p = 0.01$ ). Manure applied at rate of 180 Mg·ha<sup>-1</sup> exponentially reduced Zn levels in the soils at 0–15 cm depth ( $R^2 = 0.96$ ,  $p = 0.001$ ). Control (Mi0) Zn levels for irrigated 0–15 and 15–30 cm depths were 10.65 and 2.49 mg·kg<sup>-1</sup>, respectively. The estimated time to reduce the legacy effects to those control values at 0–15 cm depth for 60, 120, and 180 Mg·ha<sup>-1</sup> manure rates were 32, 32, and 41 yr, respectively, whereas times for the 15–30 cm depth were 23, 26, and 25 yr, respectively. Therefore, 23–41 yr are needed for Zn levels to recover from manure loadings received to irrigated fields, depending on rate of manure applied and soil depth. In rainfed treatments, the 0–15 cm depth for 30 Mg·ha<sup>-1</sup> ( $R^2 = 0.80$ ,  $p = 0.01$ ), 60 Mg·ha<sup>-1</sup> ( $R^2 = 0.60$ ,  $p = 0.05$ ) and 90 Mg·ha<sup>-1</sup> ( $R^2 = 0.79$ ,  $p = 0.01$ ) had significant linear relationships (Fig. 4). The estimated recovery times were 21, 32 and 26 yr for 30, 60, and 90 Mg·ha<sup>-1</sup>, respectively, to reach its control Zn

**Fig. 4.** Relationships between years after discontinuation of manure application with zinc (Zn) in soil under irrigated and rainfed treatments at 0–15 and 15–30 cm depths (\*\*\*, \*\*, and \* represent significant relationships at 0.001, 0.01, and 0.05, respectively; equations are given only for significant relationships; control (Mi0) Zn levels for irrigated 0–15 and 15–30 cm depths were 10.65 and 2.49 mg·kg<sup>-1</sup>, respectively, and for rainfed 0–15 and 15–30 cm were 7.15 and 1.04 mg·kg<sup>-1</sup>, respectively).



concentration of 7.15 mg·kg<sup>-1</sup> at 0–15 cm depth. Although irrigated treatments received double the Zn loadings as rainfed, the recovery times are less than double, largely reflecting greater Zn removal through crop yield and other environmental losses like leaching under irrigated conditions.

## Conclusions

In this long-term manure experiment, the higher the manure loads received by soils, the higher their DTPA-extractable Cu and Zn, with greater accumulations in the 0–15 than the 15–30 cm depth. Copper residual effects generally ended, 10–20 yr after manure discontinuation in irrigated treatments that received 30 yr of manure at different rates. The residual effect for Zn was significant 15 yr after discontinuation following 30 yr of manure applications. Results show that soil Zn concentrations declined with repeated harvesting when manure applications ceased. Zinc-enriched soils due to 14–30 yr of manure applications will take

23–41 yr to decrease to background levels under irrigated conditions, depending on the application rate.

## Acknowledgements

Funding for this research was provided by Agriculture and Agri-Food Canada (J-000251). Special thanks to Drs. T. Sommerfeldt and C. Chang for initiating and maintaining the long-term manure plot, past and current technical staffs for field sampling and laboratory analysis and field crew for seeding and harvesting over the past 45 yr.

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