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The use of N HNO3 to determine copper and zinc levels in heavy-metal polluted tropical soils

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Abstract. This study compared N HNO$_3$ to other methods to determine plant available heavy metals in heavy-metal polluted soils. Soil samples were obtained from an experimental field treated with industrial waste after 22 years of the amendment and employed to conduct the comparative and correlation study. Soil samples were analyzed for Cu using various methods, planted in a glass house with several plants, and analyzed for soil and plant Cu and Zn. The relative strength of the chemical extractants followed the order of N HNO$_3 \approx N$ HCl > Buffered DTPA $\approx$ Unbuffered DTPA > M CaCl$_2 \approx N$ NH$_4$OAc pH 7. A high correlation was observed for soil extracted Cu by M CaCl$_2$ or N NH$_4$OAc pH 7 or N HCl vs. Buffered DTPA and N HNO$_3$ or N NH$_4$OAc pH 7 vs. N HCl. High correlations of plant and soil Cu extracted by N HNO$_3$ were shown by caisim, water spinach, land spinach, and corn, while plant and soil Zn were shown by caisim, water spinach, land spinach, and lettuce.

1. Introduction

Heavy metals in soils exist in various forms, including dissolved heavy metals, adsorbed heavy metals, and structural heavy metals in soil minerals and organics [1-5]. Dissolved heavy metals include free ions, complexes, and chelates, which increase with increasing concentrations of chelating and complexing agents [2,4], generally maintained by the soil exchangeable heavy metals. Plant available heavy metals may include dissolved and adsorbed heavy metals and part of the structural heavy metals in soil minerals and organics [1,3,5]. The plant available heavy metals are much higher than the exchangeable and dissolved heavy metals but lower than the total heavy metals. For example, total Cr, Cu, and Pb in soils increased with increasing composted sewage sludge treatment rates. At the same time, their exchangeable forms were much lower, and their bio-availabilities increased with composted sewage sludge treatment, well correlated with the total organic C [2].

Therefore, the analytical techniques to determine heavy metals in soils depend on which heavy metal forms to measure [1-6]. Water extractant is generally used to determine dissolved heavy metals in soil solution [4,7]. In contrast, salt solutions like N NH$_4$OAc, M CaCl$_2$, M Sr(NO$_3$)$_2$ are commonly used to determine the exchangeable heavy metals like exchangeable Cu and Zn [4,7]. Chelating agents like EDTA and DTPA and dilute acids like N HNO3 and N HCl extract parts of soluble soil precipitates or secondary minerals. Therefore they may result in much higher concentrations of extracted heavy metals.

Acids may also extract heavy metals in soil solution and soil exchange sites [4,7] unless the soil solution and exchangeable heavy metals are first extracted before acid extraction. However, the concentration of heavy metals in soil solution is generally lower than that of exchangeable heavy metals and much lower than that in the soil mineral and organic structure [2-4,7]. The concentrations of soluble heavy metal are also dependent on soil pH [4,7-8], which are generally higher in acid soils than in alkaline soils.

The heavy metal analytical methods are of great importance related to the ever-accumulating heavy metals in the soil environment to determine their bio-accessibility, directly related to human health through food chains. Researchers from various places and disciplines reported that heavy metals were continuously accumulating in the soil environment and threatening the living things [9-19]. However, there is no suitable method currently available for tropical soils.

This research was to study N HNO$_3$ compared to several other methods to determine plant available heavy metals in more than 20 years old heavy-metal polluted tropical soils. The commonly used routine method for heavy metal analysis employing DTPA was developed for alkaline soils. In soils, soluble heavy metals were assumed to be predominantly controlled by precipitation-dissolution reactions, which is probably not suitable for tropical soils [20]. Tropical soils are unique because they are relatively acid and contain considerable amounts of variable-charged secondary minerals that affect the concentrations of heavy metals in various forms, including the soluble, exchangeable, and structural heavy metals adsorption-desorption reaction [8].

2. Materials and methods

2.1. Soil samples

A relatively wide range of heavy metal concentrations in soils was needed. Soil samples were then collected from an old and well-maintained experimental field amended with a high Cu and Zn containing industrial waste [7,21]. The initial properties of the soil and industrial waste are listed in Table 1. The soil was relatively acid with a textural class of sandy clay loam. The treatment factors and levels [21] and the treatment units are listed in Table 2. Soil samples were taken with an auger from 0 – 15 cm. The soil samples were air-dried, ground to pass a-2 mm sieve, and thoroughly mixed before being used in the experiments. All experiments and soil analysis were conducted on an oven-dry (105°C for 24 hours) equivalent basis.

Table 1. The selected initial properties of the soil and industrial waste used in this research.

Materials	Soil Fractions (Hydrometer)			pH 1:2 (H$_2$O)	Org. C (Walkley and Black)	Heavy Metals (DTPA)			
	Sand	Silt	Clay			Cu	Zn	Pb	Cd
	…..…… % ……......…				.. g kg^{-1}..	……………..…. mg kg^{-1} …………….…			
Soil[a]	41.2	26.0	32.8	5.11	1.28	2.51	1.31	0.13	0.01
Waste	-	-	-	7.30	-	754	44.6	2.44	0.12

[a]The soil textural class was Sandy Clay Loam [7]

2.2. Comparative study of several extractants

Some soil extractants were compared in extracting Cu from all soil samples (Table 2). These extractants include those that are presumably capable of extracting the exchangeable soil Cu, including N NH$_4$OAc pH 7 and M CaCl$_2$. Those capable of extracting higher exchangeable Cu including unbuffered 0.05 M DTPA (Diethylene Triamine Pentaacetic Acid) and Buffered 0.05 M DTPA, and those capable of dissolving secondary minerals, including acids of N HCl and N HNO$_3$.

The analysis followed the following steps. As much as 10 g (105°C 24 hours oven-dry equivalent) was put in an extracting bottle. After adding a 20 ml extracting solution and capped, the bottle was placed in an end-to-end shaker. After 2 hours, the soil mixture was filtered through a Whatman no. 42

filter paper. The concentration of Cu in the supernatant was then determined using flame AAS at λ = 324.7 nm.

Table 2. The existing treatment units in the experimental field at Sidosari, Natar, Lampung, Indonesia

Waste/W	Lime /L	Compost/C	
		C_0	C_1
W_0	L_0	$W_0L_0C_0$	$W_0L_0C_1$
	L_1	$W_0L_1C_0$	$W_0L_1C_1$
W_1	L_0	$W_1L_0C_0$	$W_1L_0C_1$
	L_1	$W_1L_1C_0$	$W_1L_1C_1$
W_2	L_0	$W_2L_0C_0$	$W_2L_0C_1$
	L_1	$W_2L_1C_0$	$W_2L_1C_1$

Notes:
W = Waste (W_0 0, W_1 15, and W_2 60 Mg ha^{-1}), L = Lime (L_0 0 and L_1 5 Mg CaCO$_3$ ha^{-1}); and C = Cassava-Leaf Compost (C_0 0 and C_1 5 Mg ha^{-1}); after [21]

2.3. Correlation study
A 200 g (105°C, 24 hours oven-dry equivalent) soil sample was used as a planting medium in a glass-house. Seeds or seedlings of a particular plant were planted in each pot, and one seedling was left in the soil medium after one week. This plant was let to grow for 4 weeks. The soil water content was capillary maintained at the soil field-water capacity by a common water reservoir beneath pots during the planting growth. Plants investigated included caisim (*Brassica chinensis*), water spinach (*Ipomoea aquatica*), land spinach (*Ipomoea reptans*), lettuce (*Lectuca sativa*), and corn (*Zea mays*).

Plant and soil samples were harvested after a four-week plant growth. Each plant was cut at the soil surface. Plant biomasses were carefully cleaned from the soil masses using tap water. Plant biomasses were weighed for their dry-weights after being oven-dried at 60°C for 3×24 hours and analyzed for Cu and Zn. Soil samples were also harvested and analyzed for Cu and Zn

One gram of oven-dried and ground plant tissue was put in a porcelain crucible and placed in a furnace to analyze the concentration of Cu and Zn in plant roots and plant shoots. It heated at 300°C for 2 hours and then at 500°C for 4 hours, after which the plant sample was let to reach room temperature [22]. The plant sample was wetted with several drops of distilled water, treated with 10 ml of 1 N HCl, put on a hot plate, and let to boil gently. After cooling, the soluble plant tissue ash was filtered into a 100 ml volumetric flask. The crucible was then rinsed with 10 ml 1 *N* HCl, and about 50 ml distilled water on the filter paper into the volumetric flask. Distilled water was added to dilute the filtrate to 100 ml. The filtrate was gently shaken before analysis. The extraction of the soil Cu and Zn used 1 *N* HNO$_3$. Cu and Zn concentrations in the filtrates were determined by flame AAS at λ = 324.7 nm for Cu and λ = 213.9 nm for Zn.

3. Results and discussion

3.1. The relative strength of various extractants
The extractability of Cu from soils with different treatment levels of waste, lime, and cassava-leaf compost in 1998 or 20 years before soil sampling in 2018 by different soil heavy-metal extractants is shown in Table 3. The soil Cu extractability follows the order of those extracted by *N* HNO$_3$ (34.0-54.9 mg kg^{-1}), *N* HCl (19.0-63.4 mg kg^{-1}), Unbuffered DTPA (9.55-40.3 mg kg^{-1}), Buffered DTPA (7.56-29.0 mg kg^{-1}), *N* NH$_4$OAc (0.12-1.83 mg kg^{-1}) and *M* CaCl$_2$ (0.16-0.86 mg kg^{-1}). The highest concentration for each extractant was theoretically found in waste amended soil not treated with lime and cassava-leaf compost. The lowest was in control soil treated with lime and cassava-leaf compost. Lime and cassava-leaf compost were reported to lower the concentrations of heavy metals [7,23,24]. However, our data do not clearly show this phenomenon. The organic matter oxidation increased the

heavy metal mobilization and bio-availability of soil heavy metals. The lime effect may have attenuated with time [25]. In the last 20 years, soil tillage was also suspected of moving heavy metals beyond plot boundaries to nearby plots, including the control plots [26].

Table 3. The extractability of Cu from 20 years old heavy-metal polluted tropical soil by several chemical extractants.

1998 Treatment			Buffered DTPA	Unbuffered DTPA	N HNO$_3$	N HCl	N NH$_4$OAc	M CaCl$_2$
Waste[a]	Compost[b]	Lime[c]						
………….. Mg ha^{-1} ……..			…………….……….…...… mg kg^{-1} ……..……………….…………..					
0	0	0	7.56	13.2	54.6	27.5	0.29	0.24
	0	5	23.7	16.7	54.9	47.8	0.65	0.79
	5	0	10.0	16.0	44.0	51.3	0.34	0.86
	5	5	12.8	9.55	39.2	22.7	0.12	0.19
15	0	0	21.6	40.3	34.0	19.0	1.12	0.73
	0	5	8.77	20.6	43.5	40.2	0.18	0.16
	5	0	8.39	24.1	41.0	33.8	0.88	0.18
	5	5	17.7	16.3	47.4	41.5	0.26	0.51
60	0	0	29.0	24.4	47.8	36.0	1.83	0.33
	0	5	18.6	34.2	47.8	35.2	0.67	0.59
	5	0	17.7	19.1	39.0	63.4	0.40	0.35
	5	5	7.96	27.6	41.7	33.4	0.16	0.20

[a]Waste of metal wares industry, [b]cassava-leaf compost, [c]CaCO$_3$,

The higher extracted Cu by acids, i.e., N HNO$_3$ and N HCl is as expected. Dilute acids extracted the dissolved and exchangeable heavy metals and the highly soluble (high K_{sp}) heavy-metal precipitates. These secondary minerals may readily dissolve in dilute acids releasing their structural heavy metals. The dilute salts, as usual, extract only the soluble heavy metals and the exchangeable heavy metals. M CaCl$_2$ must extract more heavy metals than N NH$_4$OAc because Ca^{2+} possesses 2 positive charges while NH$_4^+$ has only one positive charge. However, M CaCl$_2$ extracted a lower amount of Cu than N NH$_4$OAc. The higher pH of M CaCl$_2$ than N NH$_4$OAc may explain this phenomenon (Table 4). However, DTPA extracted more Cu since DTPA may act as a Cu^{2+} pool that may maintain the soil concentration of Cu^{2+} low during soil extraction and stimulate greater Cu release from more strongly bonded Cu from the soil exchange sites. Therefore, the relative strength of DTPA is higher than dilute salts (N NH$_4$OAc and M CaCl$_2$) but lower than those dilute acids (N HCl and N HNO$_3$) (table 4).

Table 4. The reaction and extraction power of the chemical extractants

Extractants	pH	Average Extracted Cu[a] (mg kg^{-1})	Relative Strength[b]	Probable Cu Forms Extracted
M CaCl$_2$	7.38	0.46	0.03	Dissolved Cu, Cu-Complexes/Chelates, Exchangeable Cu
N NH$_4$OAc pH 7	7.00	0.63	0.04	Dissolved Cu, Cu-Complexes/Chelates, Exchangeable Cu
Buffered DTPA	7.30	15.3	1.00	Dissolved Cu, Cu-Complexes/Chelates, Exchangeable Cu, High K_{sp}[c] Cu Minerals
Unbuffered DTPA	8.14	20.9	1.37	Dissolved Cu, Cu-Complexes/Chelates, Exchangeable Cu, High K_{sp} Cu Minerals
N HCl	1.38	38.9	2.54	Dissolved Cu, Cu-Complexes/Chelates, Exchangeable Cu, High K_{sp} Cu Minerals, Low K_{sp} Cu Minerals
N HNO$_3$	0.72	44.3	2.90	Dissolved Cu, Cu-Complexes/Chelates, Exchangeable Cu, High K_{sp} Cu Minerals, Low K_{sp} Cu Minerals

[a]Average of all soil samples, [b]Relative Strength = Extractability relative to that by Buffered M DTPA, [c]solubility product
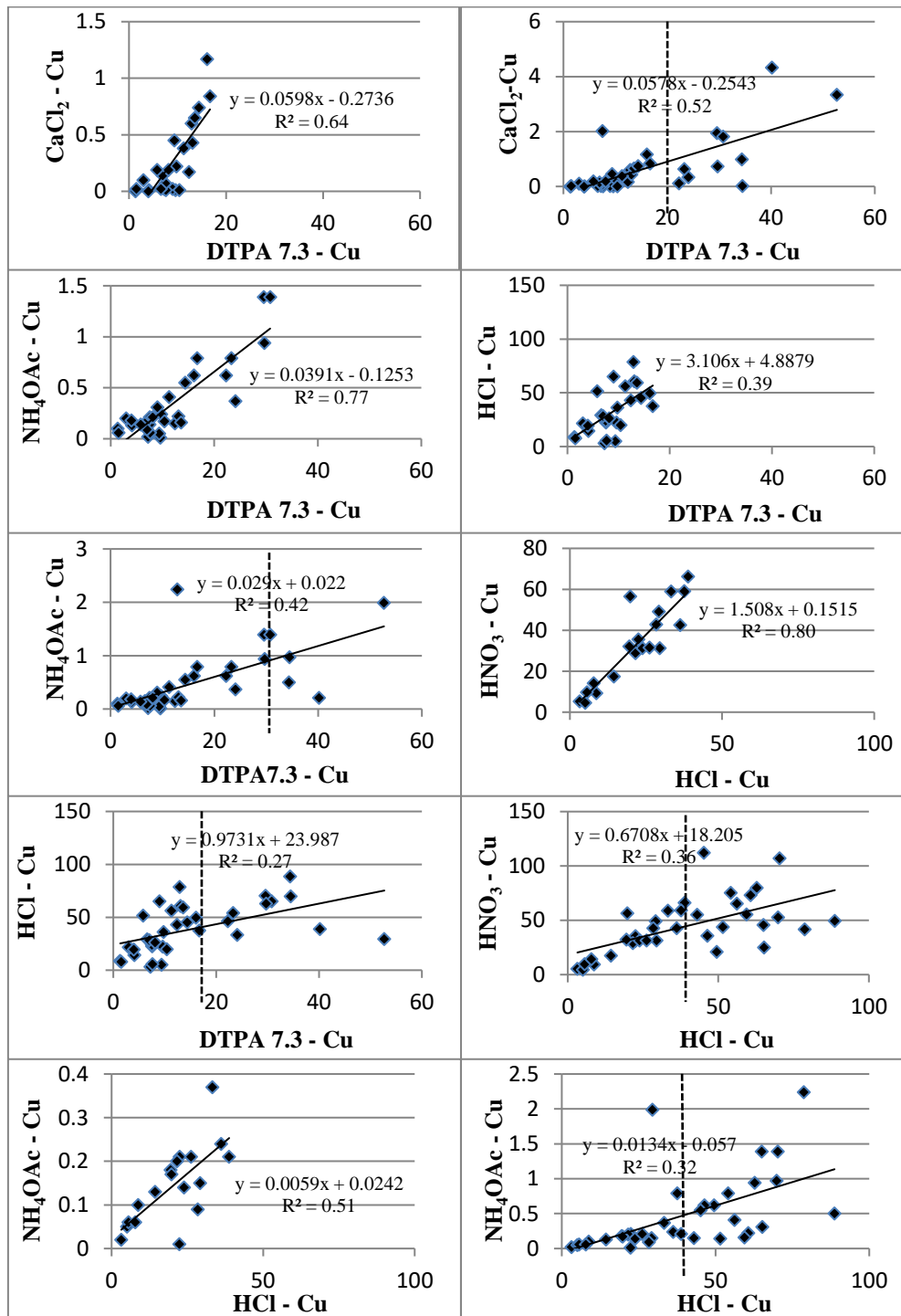
Figure 1. The correlations of several chemical extractants for Cu levels in polluted tropical soils.

The above phenomenon is related to the relative strength of each chemical extractant which follows the order of N HNO$_3$ > N HCl > Unbuffered DTPA > Buffered DTPA > N NH$_4$OAc pH 7 > M CaCl$_2$ (Table 4). Relative strength is the ability to extract heavy metals by a particular method divided by that which can be extracted with Buffered DTPA. As seen in Table 4, the dilute salts of CaCl$_2$ and NH$_4$OAc extracted only the soluble and exchangeable heavy metals. At the same time, chelates like DTPA were

able to extract also the more strongly held heavy metals by soil minerals, and the dilute acids also extract heavy metals from lower K_{sp} soil minerals.

3.2. The relationship among chemical extractants

Due to the possible forms of soil heavy metals that various extractants can extract, different extractants' ability to extract heavy metals in soil is well correlated (R^2 ranges from 027 – 0.52; Figure 1).

The correlation coefficients improve to $R^2 = 0.39 – 0.80$ if the range of soil heavy metals considered were narrower, about 0 – 20 or 0-50 mg kg^{-1}, dependent on the extractant instead of 0 – 100 mg kg^{-1}. The changes in R^2 are listed in table 5. For example, the relationship between the extractability of N NH$_4$OAc and Buffered DTPA improves from 0.42 to 0.76 if the heavy metal range was narrowed from $0 – < 60$ mg kg^{-1} to < 30 mg kg^{-1} (figure 1, table 5). The correlation coefficients of the extractability N HNO$_3$ vs N HCl improves from 0.36 to 0.80 if the heavy metal range is narrowed from 0 to < 100 mg kg^{-1} to $0 - < 40$ mg kg^{-1} (figure 1, table 5). These observations show that all these methods are good predictors for plant heavy metal availability in soils. However, they need correlations studies.

3.3. The correlation of plant and soil heavy-metals

The correlation studies with several plants are depicted in figure 2, which shows the relationships between plant accumulation and soil concentration of Cu extracted by N HNO3. Figure 3 shows the relationship between plant accumulation and the soil concentrations of Zn extracted by N HNO$_3$. High correlations were found in caisim ($R^2 = 0.71*$), water spinach ($R^2 = 0.32*$), land spinach ($R^2 = 0.87*$), and corn ($R^2 = 0.66*$) for Cu and in caisim ($R^2 = 0.96*$), water spinach ($R^2 = 0.95*$), land spinach ($R^2 = 0.78*$), and lettuce ($R^2 = 0.64$) for Zn.
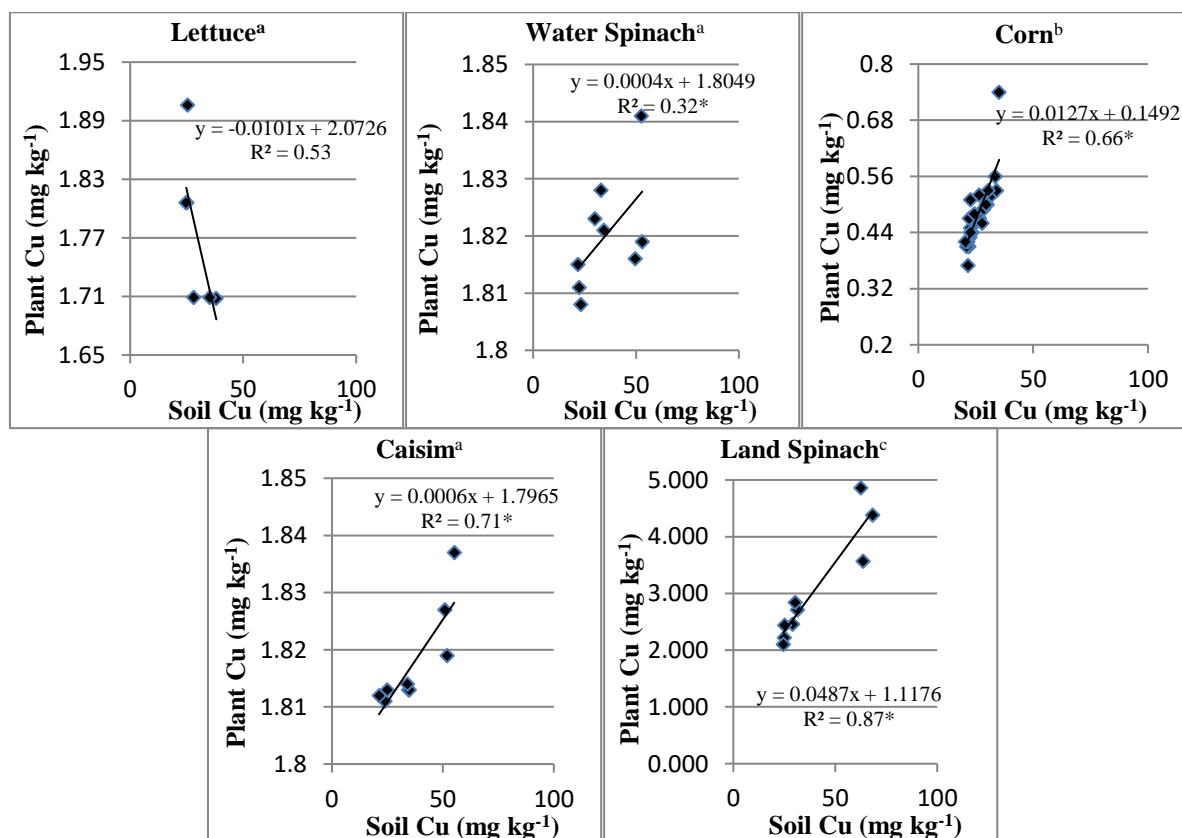

Figure 2. The relationship between Cu uptake and N HNO$_3$ extracted Cu from soil planted with several plants ([a]after [22], [b]Salam (Unpublished Data), [c]Rachman and Salam (Unpublished data)).

These observations show that N HNO$_3$ was a good extractant for soil Cu and Zn and can predict the plant uptake of Cu and Zn for these plants. The accurate prediction was in the Cu and Zn concentration between 0 – 60 mg kg^{-1}. However, further research is needed to employ more dilute HNO$_3$ to match the Cu and Zn accumulation in a particular plant.

Table 5. The improvement of the correlation coefficients (R^2) for several extractants for soil Cu.

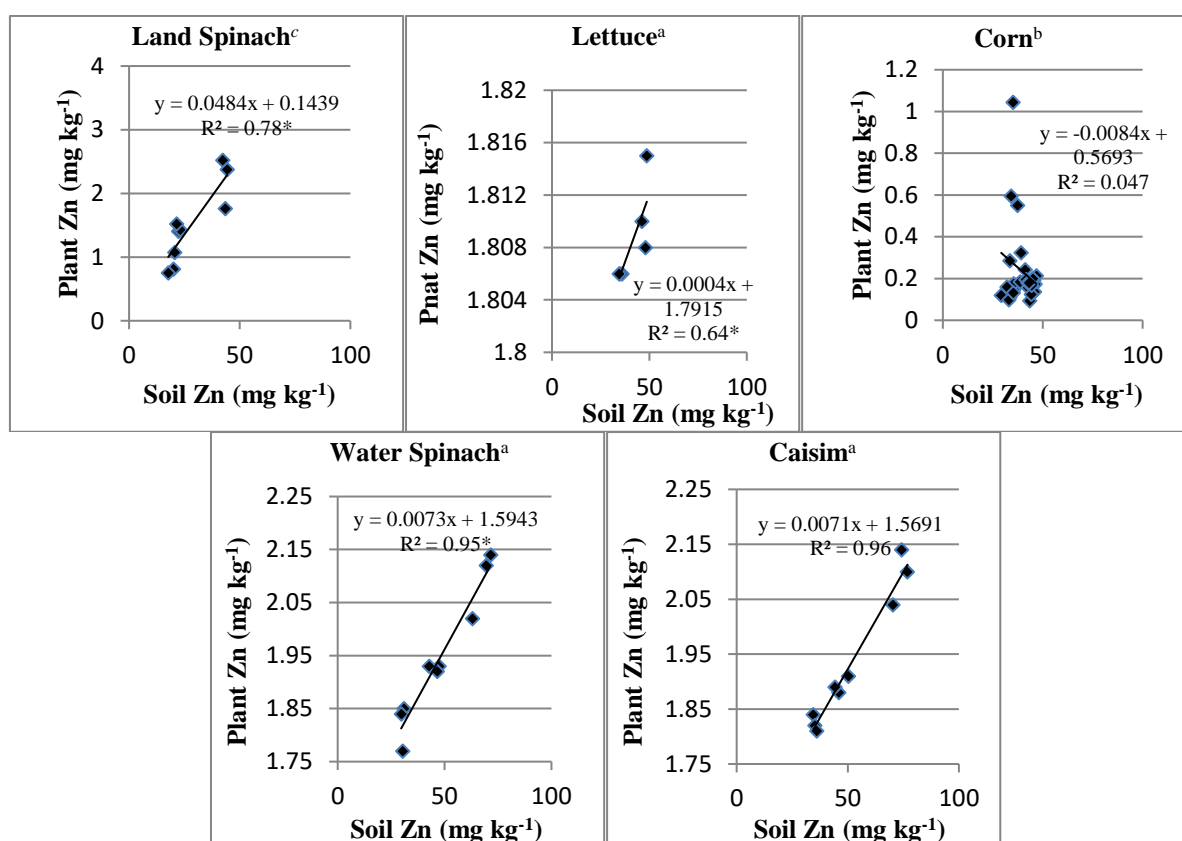| Extractant 1	Extractant 2	High Concentrations		Lower Concentrations	
		mg kg^{-1}	R^2	mg kg^{-1}	R^2
M CaCl$_2$	Buffered DTPA	< 60	0.52	< 20	0.64
N NH$_4$OAc pH 7	Buffered DTPA	< 60	0.42	< 30	0.76
N HCl	Buffered DTPA	< 60	0.27	< 20	0.39
N HNO$_3$	N HCl	< 100	0.36	< 40	0.80
N NH$_4$OAc pH 7	N HCl	< 100	0.32	< 40	0.51

Figure 3. The relationship between Zn uptake and HNO$_3$ extracted Zn from soil planted with several plants ([a]Silva and Salam (Unpublished Data), [b]Salam (Unpublished Data), [c]Rachman and Salam (Unpublished Data)).

4. Conclusions

The relative strength of the chemical extractants follows the order of 1 N HNO$_3$ ≈ 1 N HCl >> Buffered DTPA ≈ Unbuffered DTPA > N NH$_4$OAc pH 7 ≈ M CaCl$_2$. A high correlation was observed for soil extracted Cu by M CaCl$_2$ or N NH$_4$OAc pH 7 or N HCl vs. Buffered DTPA and N HNO$_3$ or N NH$_4$OAc pH 7 vs. N HCl. High correlation of plant and soil Cu extracted by N HNO$_3$ was shown by caisim, water spinach, corn, and land spinach, while soil Zn was shown by caisim, water spinach, lettuce, and land spinach.

Acknowledgment

References

[1] Zimmerman A J and Weindorf D C 2010 *Int J Anal Chem* **2010** 387803
[2] Yang K *et al.* 2018 *Int J Environ Res Publ Health* **15** 613
[3] Kumar B *et al.* 2011 *Adv Appl Sci Res* **2** 263–72
[4] Salam A K 2017 *Management of Heavy Metals in Tropical Soil Enviroment*, vol 1 (Bandar Lampung: Global Madani Press)
[5] Osakwe S A and Okolie L P 2015 *J Appl Sci Environ Manag* **19** 706–16
[6] Wei M, Chen J, Sun Z, Lv C and Cai W 2015 *Prof World Congr New Technol* **2015** 200
[7] Salam A K 2000 *J Trop Soils* **11** 31–46
[8] Salam A K and Helmke P A 1998 *Geoderma* **83** 281–91
[9] Aksu A 2015 *J Environ Health Eng* **13** 79
[10] Asati A, Pichhode M and Nikhil K 2016 *Int J Appl Innov Eng Manag* **5** 56–66
[11] Elbagermi M A, Edwards H G M and Alajtal A I 2013 *Int J Anal Chem* **2013** 18–23
[12] Febriansyah M R, Septiana L M, Supriatin S and Salam A K 2021 *IOP Conf Ser: Earth Environ Sci* **739** 012001
[13] Gill M and Gill M 2014 *Int J Adv Res* **2** 1043–55
[14] Jamal Q, Khan K, Munir S and Anees M 2013 *J Biomol Sci* **1** 27–36
[15] Smolders E, Oorts K, Lombi E, Schoeters I, Zrna S and Mclaughlin M J 2012 *J Environ Qual* **41** 506–14
[16] Sun L *et al.* 2019 *Catena* **175** 101–9
[17] Wuana R A and Okieimen F E 2011 *ISRN Ecol* **2011** 402647
[18] Timothy N and Williams E T 2019 *Int J Environ Chem* **3** 72–82
[19] Popova E 2016 *AIP Conf Ser* **1772** 050006
[20] Lindsay W L and Norvell W A 1978 *Soil Sci Soc Am J* **42** 421–8
[21] Salam A K and Ginanjar K 2018 *J Trop Soils* **23** 11–8
[22] Silva G, Aini S N, Bucharie H and Salam A K 2021 *J Trop Soils* **26** 11–8
[23] Xiao R, Huang Z, Li X, Chen W, Deng Y and Han C 2017 *Sustainability* **9** 430
[24] Zhang D, Li T, Wu X and Wang Y 2021 *IOP Conf Ser: Earth Environ Sci* **742** 012016
[25] Stietiya M H and Wang J J 2011 *J Environ Qual* **40** 1162–71
[26] Yingming L and Corey R B 1993 *J Environ Qual* **22** 1–8