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Pyroligneous acid influenced the bioavailability of heavy metals in contaminated paddy soil

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ABSTRACT

Utilization of pyroligneous acid known as wood vinegar (WV) benefited crop productivity through improved growth and development by supplying nutrients and crop protection. Its effect on the mobility and availability of heavy metals in soil-plant continuum is rarely studied therefore its potential as an organic amendment for ameliorating contaminated paddy soils was evaluated. Pot experiments using heavy metal spiked-paddy soil were conducted with the treatments: control, contaminated soil (CS); CS + 100x–WV, CS + 250x–WV, and CS + 500x–WV. Plant growth and yield parameters in rice were promoted by WV application at 500-x dilution. All WV treatments have comparable effects on Cu, Pb, and Zn contents in rice grains which are below the permissible values for consumption. Sequential extraction of heavy metals in soil showed the highest concentration of Cu and Zn in the residual fractions which are relatively stable and unavailable for plant uptake. A moderately high percentage of Pb and Zn in the Fe-Mn oxide fraction was observed which could be mobilized under reducing and acidic conditions. The changes in the percentage of metals in each fraction indicated that WV could displace ions and enhance mobility thus affecting the distribution in soil and plants. These findings indicate that WV (0.2%) can mitigate possible harmful effects of heavy metals and ensure food safety. Further investigation is warranted to fully understand the long-term effects of WV applications under field conditions.

Keywords: heavy metals, pyroligneous acid, rice, sequential extraction, wood vinegar.

INTRODUCTION

Land is one of the most significant, non-renewable geo-resource in agriculture. On a global perspective, about 21% (2.74 billion ha) of the total land area (13.5 billion ha) are considered as marginal lands. However, about 1,558 million ha of these lands are devoted to agriculture, wherein about 224 to 300 million ha is classified as agriculturally marginal areas (Ahmadzai et al., 2022). These lands are considered having limitations which in aggregate are severe for sustained application of a given use and will so reduce productivity or benefits, or increase required inputs, that this expenditure will be only marginally justified. Some agricultural soils are already characterized as slightly contaminated soils because of contamination from fertilizer or pesticide application, mine tailing or air deposition. The presence of heavy metals in the soil is a limiting factor and also one of the most prevalent environmental problems agriculture is facing today.

One of the commonly used methods for toxicity alleviation of soils contaminated with heavy metals is the application of organic amendments. Aside from its beneficial effects in enhancing crop production (Chiu et al., 2006), organic amendments such as compost, farm manure and organic wastes are known to immobilize metals and rehabilitate contaminated soils (Clemente et al., 2005). The use of pyroligneous acids, also known as wood vinegar, has gained interest due to the number of beneficial effects it can contribute. This pyrolysis-based product has diverse functions which include antimicrobial (Jain et al., 2007), antioxidant (Cai et al., 2012) and pesticidal properties (Kim et al., 2008), and contain growth-stimulating substances (Mu et al. 2006) that can improve productivity in agricultural crops.

India, China and Indonesia are the main countries whose lands are devoted in planting rice. As of 2022, the world's total land area planted to rice is 165.69 M hectares. When it comes to production of milled rice, China comes first at about 145.95 M metric tons (USDA, 2024). Various studies have shown the beneficial effects of applying pyroligneous extract applied to soil as an organic fertilizer for growing rice (Wanderley et al., 2012). However, where contaminated agricultural soils are concerned, literature is limited.

Heavy metal contamination of food chain causing adverse effects on human health also warrants special attention. The problematic heavy metals as cited by Ali et al. (2013) include mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), copper (Cu), zinc (Zn), tin (Sn), and chromium (Cr). This study focused on the prevalent heavy metals affecting agricultural soils which include Cd, Cu, Pb and Zn. These metals also belong to the most common problem causing cationic metals. The total metal content in soil is helpful in terms of geochemical applications but it is the bioavailability and mobility of these metals which is the major concern in agriculture. Therefore, fractionation through sequential extraction is necessary. Metal fractionation or speciation is the identification and quantification of the various metal species, forms, or phases in which an element occurs (Tack and Verlo, 1995).

Given the benefits derived from wood vinegar applications, investigating the wood vinegar-heavy metal interaction in contaminated agricultural lands, its effect on metal speciation, and its influence on crop growth are the objectives of this study.

MATERIALS AND METHODS

A greenhouse experiment consisting of five treatments with three replications was conducted in a complete randomized design. The treatments include control [no wood vinegar (WV) & no heavy metal solution (HMS)]; contaminated soil (CS, with HMS); contaminated soil + 100 times diluted wood vinegar (CS + 100x-WV); CS + 250x-WV; and CS + 500x-WV. The set-up was terminated at maturity.

Planting and treatment applications

Pre-sown rice seeds were allowed to grow for one month before transplanting into bigger pots. The treatment application was done after seedling establishment. One plant per pot was maintained throughout the growing period. Soil properties and wood vinegar characteristics were analyzed and are presented in Tables 1 and 2, respectively. Heavy metal solution was prepared using the following reagents and concentrations. Five hundred ml of the solution was delivered on designated pots. The concentration used was adopted from the research of Kabata-Pendias (2011) which was also cited by Aziz et al., (2023). The values used were also within the range of warning levels set under Korean paddy soils (Yoon, 2015).

1. Lead nitrate; 400 mg kg^{-1} Pb
2. Copper chloride dehydrate; 100 mg kg^{-1} Cu
3. Cadmium chloride hemi pentahydrate; 30 mg kg^{-1}m Cd
4. Zinc chloride; 200 mg kg^{-1} Zn

Wood vinegar was obtained from the Korean Ministry of Agriculture and Forestry. The pyroligneous acid was prepared from a liquid condensate extracted between 80 °C to 120 °C during the carbonization process of Korean oak tree (*Quercus* sp.). The condensate was aged for 3 months and the thick liquid was collected. The same process was repeated before the commercial quality wood vinegar was collected (Jeong, et al., 2015). Wood vinegar treatments were prepared by the following dilutions and were applied twice a week at 500 ml/pot: 100-x dilution (10 ml WV/li), 250-x dilution (4 ml WV/li), and 500-x dilution (2 ml WV/li).

Data gathering on agronomic parameters

The data on plant height of rice commenced five weeks after transplanting (WAT) until 11 WAT. Fresh and oven-dried weights of roots and shoots, number of panicles, number of filled grains, and weight of filled grains were recorded.

Table 1. Selected chemical properties of soil

pH	Parameter	7.78
EC	*uS*/cm	413.75
Exchangeable bases	$cmol_c$ kg^{-1}	
Calcium		11.14
Magnesium		3.43
Potassium		0.33
Sodium		1.09
Mehlich 3 extractable phosphorous	mg kg^{-1}	3118.95
Heavy metals	mg kg^{-1}	
Cadmium		ND
Copper		20.58
Lead		32.77
Zinc		82.01

Table 2. Physicochemical characteristics of wood vinegar

pH	Parameter	2.97
Electrical conductivity	*uS* cm^{-1}	2313
Methanol	%	0.31
Phenolic compounds	%	2.44
Propanoic acid	%	0.89
Acetic acid	%	9.95
Total amino acids	*ug* ml^{-1}	38.89
Ammonia	*ug* ml^{-1}	471.552
Calcium	mg li^{-1}	19.71
Magnesium	mg li^{-1}	0.88
Potassium	mg li^{-1}	5.153
Phosphorous	mg li^{-1}	2.42
Sulfur	mg li^{-1}	41.63
Boron	mg li^{-1}	0.27
Lead	mg li^{-1}	1.20
Zinc	mg li^{-1}	0.68
Cadmium	mg li^{-1}	ND
Copper	mg li^{-1}	ND
Transparency		Transparent
Color		Golden brown

Heavy metal analysis in plant tissues

Oven-dried samples were grinded and sieved using fine mesh (5 mm) then the modified wet digestion method cited by Huang et al. (2013) was used for pretreatment of plant samples. A flask containing 0.2–0.5 g sample was added with 5 ml concentrated nitric acid (HNO_3) then covered with funnel. The samples were allowed to stand overnight or until the fizzing recedes. Samples were heated on a hot plate (120 °C) for 1 hour then were allowed to cool before adding 2 ml of 30% hydrogen peroxide (H_2O_2). The samples were again reheated at 120 °C and H_2O_2 was continuously added until digest is clear. Nitric acid was added to maintain a wet digest. When digest is clear, the temperature was lowered to 80 °C. Continuous heating was done up to near dryness or appearance of clear/white residues was attained. Samples were cooled then diluted with distilled water at a desired volume. The samples were analyzed for presence of Cd, Cu, Pb, and Zn in plant tissues using Inductively Coupled Plasma Spectrophotometer (ICP).

Soil pH determination

Collected soil samples were air dried and pulverized then allowed to pass through a fine mesh (2 mm) prior to analysis. The method cited by Rayment and Higginson (1992) was used in measuring soil pH. A 1:5 (soil : water) suspension was prepared then incubated for 1 hour with continuous shaking (15 rpm). Soil pH was measured using an electrode-multi parameter analyzer (Thermo Scientific Orion Star A215).

Sequential extraction of heavy metals in soil

Same preparation of soil samples as those in pH determination were done for sequential extraction of heavy metals in soil. Sequential extraction for metal fractionation or distribution in soil was determined following the procedure cited by Yusuf (2007) which is a modified version of Tessier sequential chemical extraction scheme (supplementary data provided). It is designed to separate heavy metals into five operationally defined fractions.

Before proceeding to the next extraction step, the residues were washed with 8 mL of distilled water, followed by 30 minutes of centrifugation, then the wash solution was discarded. After each successive extraction, the mixture was centrifuged at 3000 rpm for 30 minutes and the supernatant decanted into polypropylene bottles, acidified to pH < 2 and stored at 4 °C before analysis. All pH adjustments were done using HNO_3. The Cd, Cu, Pb and Zn contents of the five fractions in filtered solution were determined by ICP. The amount of extracted trace metals in each fraction, as a percentage of total metal content was also calculated.

Bioconcentration factor and translocation factor

To evaluate the transfer of heavy metals in soil-plant system as affected by wood vinegar, the bioconcentration factor (BCF) and translocation factor (TF) of metals were determined. These parameters serve as a useful indicator of availability of heavy metals from soil to plants. BCF and TF were calculated following the formula cited by Ali et al. (2013):

$$BCF = [metals]\ root/[metals]soil \quad (1)$$

$$TF = [metals]\ aerial\ parts/[metals]root \quad (2)$$

Correlation between heavy metals in plants and soil

To determine the relationship between heavy metals in plants and those in different soil fractions, correlation analyses involving the four metals were performed.

Statistical analysis

Experimental data were analyzed using the analysis of variance (ANOVA) procedure of the Statistical Tool for Agricultural Research Software (STAR) Version 2.0.1. The mean separation method used was the Tukey's Honest Significant Difference (HSD) at 5% significance level.

RESULTS AND DISCUSSION

Growth and yield of rice as affected by wood vinegar

The plant height of rice was monitored 5–11 weeks after transplanting. Significant differences were observed in the weekly monitoring of plant height which indicates that it was significantly enhanced by wood vinegar applications using 250-x and 500-x dilutions. Moreover, wood vinegar at the lowest concentration (diluted 500x) effected the highest increase in plant height compared to other treatments. The percentage increase was 14.06%, 8.42%, and 18.02% over the Control, CS, and CS + 100-x WV, respectively.

The effect of heavy metal and wood vinegar application on the biomass parameters of rice are presented in Table 3. Wood vinegar application on contaminated soil (CS) at 500-x dilution provided an increase in the root fresh weight (6.10%), root dry weight (9.16%), and shoot fresh weight (1.0%) while an inhibitory effect was seen on these parameters with wood vinegar application at 100-x. Alternatively, shoot dry weight was favored by wood vinegar application at 100-x dilution (8.53% increase).

Table 4 shows the number of panicles, number of filled grains, and weight of filled grains. Heavy metal solution applied in soil generally reduced panicle formation as seen in the number of panicles. When wood vinegar was applied (100-x dilution) a comparable increase in panicle number over the positive control (CS treated plants) were observed. Moreover, the number and weight of filled grains benefitted more when a lower concentration (500-x) of wood vinegar was applied.

Crop growth and yield was evaluated based on plant height, fresh and dry weights of roots and shoots, number of panicles, and number and weight of filled grains. The application of wood vinegar in contaminated soil at the lowest concentration (500-x dilution) generally promoted these parameters in rice. A study conducted by Tsuzuki et al. (1989) showed that application of wood vinegar in rice under field condition increased root dry weight and promoted root respiration rate. Plant height, root length, and branching

Table 3. Biomass parameters of rice as affected by wood vinegar application in contaminated soil

Treatment/Variable	Fresh weight (g)		Dry weight (g)	
	Root	Shoot	Root	Shoot
Control	20.26 ab	181.63 ab	10.82 bc	40.95 b
CS	21.99 ab	191.52 a	11.36 ab	43.14 ab
CS + 100-x WV	18.97 b	185.14 ab	9.71 c	46.82 a
CS + 250-x WV	20.04 ab	173.93 b	11.96 ab	40.08 b
CS + 500-x WV	23.33 a	193.24 a	12.40 a	40.69 b

Note: Values within a parameter followed by the same letter(s) are not significantly different among treatments at 5% level based on Tukey's HSD test.

Table 4. Influence of wood vinegar application on number of panicles, number of filled grains, and weight of filled grains of rice planted in contaminated soil

Treatment/Variable	No. of panicles	No. of filled grains	Weight of filled grains (g)
Control	43 a	1681 b	42.02 a
CS	38 bc	1952 a	43.21 a
CS + 100-x WV	41 ab	1758 ab	39.96 a
CS + 250-x WV	37 c	1817 ab	39.98 a
CS + 500-x WV	38 bc	1957 a	45.38 a

Note: Values within a parameter followed by the same letter(s) are not significantly different among treatments at 5% level based on Tukey's HSD test.

were enhanced in nursery conditions. In vitro experiment on root tip culture confirmed that wood vinegar induced the formation and elongation of branched roots. They speculated that the effect might be hormonal. Their data proposed that rice growth was favored by wood vinegar through development of branched roots.

Confirmatory studies in field and laboratory conditions demonstrated that the application of wood vinegar increased the root dry weight, promoted plant height, and increased grain yield of rice (Tsuzuki et al. 2000). The beneficial effect on plants brought about by wood vinegar was because of the major components such as organic acids and phenols. Studies conducted by Kadota et al. (2002) showed that these substances are synergistic and accelerated the growth of roots in rice. Seo (2015) also mentioned in their study on rice that the improvement of plant growth in treatments with wood vinegar could be due to additional beneficial nutrients and organic substances present.

Heavy metal concentration and uptake in rice as influenced by wood vinegar

The Zn concentration was highest in all plant parts among the four metals analyzed regardless of treatment applications (Table 5). The metal contents in each plant part were observed to be in the decreasing order: roots > straw > grains. The amount of Cu in grains was generally decreased by wood vinegar applications. Moreover, the application of wood vinegar at 500-x dilution significantly decreased the Cu concentration in grains by 24%. Although comparable to the positive control, wood vinegar application at 250-x dilution also reduced Pb and Zn concentration in grains. Wood vinegar applied in contaminated soil did not show a significant effect in the amount of Cu, Pb, and Zn in rice shoots. Although insignificant, the concentration of these heavy metals was reduced by 11.76%, 21.48%, and 11.59%, respectively, when wood vinegar at 100x dilution was applied. Additionally, Zn concentration in the rice shoot was decreased more when wood vinegar at 500-x dilution was applied (17.68%). Similar to rice straw, Cu content in roots was insignificantly influenced by wood vinegar application. Nonetheless, the application of wood vinegar resulted in an increase in Pb and Zn concentrations in roots. Cadmium was below the detectable limits in all plant parts analyzed.

The highest concentration of heavy metals in roots followed by shoots then grains is a plant's response to lessening toxicity caused by heavy metals in the soil. To minimize toxicity, the translocation to the aboveground parts was also minimized. This behavior is common in plants as a response towards unfavorable conditions such as heavy metal stress (Wang et al., 2003). As cited by St-Cyr and Campbell (1996), roots of aquatic/wetland plants were found to contain higher metal concentrations than the aboveground parts. This may have something to do with the formation of Fe root plaques under anaerobic conditions which in turn might bind with other metals making them less available for uptake. Hansel and Fendorf (2001) also mentioned that these root plaques were suspected to limit the mobility and uptake of metals.

The lower concentration of Cu and Pb compared to Zn in the upper plant parts is inherent to these metals being immobile. Zinc and Cu also have the same mechanism of absorption which makes them antagonistic to each other. Copper absorption is inhibited by Zn as well as NH_4^+, Ca and K (Alloway, 1995). Ammonia in wood vinegar upon reaction with water forms ample amounts of NH_4^+ ions which may also prevent Cu absorption. In contrast, the higher concentration of Cu and Zn as compared to Pb in the grains

Table 5. Heavy metal concentration in grains, shoots, and roots of rice as affected by wood vinegar application in contaminated soil

Treatment/Variable	Cd	Cu	Pb	Zn
	(mg kg^{-1})			
Grains				
Control	-	0.53 b	0.00 b	3.85 a
CS	-	0.75 a	0.56 a	4.02 a
CS + 100-x WV	-	0.61 ab	0.57 a	4.17 a
CS + 250-x WV	-	0.62 ab	0.48 a	3.65 a
CS + 500-x WV	-	0.57 b	0.58 a	3.70 a
Shoots				
Control	-	0.00 b	0.60 a	6.92 a
CS	-	0.51 a	1.49 a	4.92 b
CS + 100-x WV	-	0.45 a	1.17 a	4.35 b
CS + 250-x WV	-	0.51 a	1.48 a	5.04 b
CS + 500-x WV	-	0.50 a	1.64 a	4.05 b
Roots				
Control	-	1.66 b	1.69 c	8.73 d
CS	-	3.55 a	4.61 b	13.31 c
CS + 100-x WV	-	3.55 a	6.43 a	12.60 c
CS + 250-x WV	-	3.51 a	6.48 a	22.18 b
CS + 500-x WV	-	3.25 a	1.10 ab	1.89 A

Note: Values within a parameter followed by the same letter(s) are not significantly different among treatments at 5% level based on Tukey's HSD test.

can be supported by the soil-plant transfer coefficients of heavy metals. The transfer coefficient is the ratio of metal concentration in above ground plant parts to that of total metal concentration in soil (Kloke et al., 1994). The transfer coefficients of Cu (0.1–10) and Zn (1–10) are much higher than that of Pb (0.01–0.1). The values are given to serve as a guide to the order of the magnitude of the transfer coefficients and not precise values since numerous soil and plant factors can affect metal accumulation in plants. These findings also supported by the data on translocation factors (Table 6). This parameter shows the ability of the plant to translocate accumulated metals from the roots to the aboveground portions of the plant (Coupe et al., 2013). The TF values for Cu were higher in grain than the straw which justifies the higher Cu concentration in grains compared in straw. Oppositely, the higher concentration of Pb and Zn in straw than in grain can also be explained by the TF values obtained in the experiment. A lower BCF value on the other hand means that lower concentration of metals was translocated in the roots.

The uptake of heavy metals was highest in roots followed by straw then grains (Table 7). Almost similar trends were observed in concentration and uptake of heavy metals in rice. A reduction in Cu uptake was attributed to the application of 500-x WV. A comparable decrease was also seen in Pb and Zn uptake through wood vinegar application at 250-x dilution. Metal uptake in straw did not exhibit noticeable response to wood vinegar application in contaminated soil. Interestingly, Pb and Zn uptake in roots were relatively higher in wood vinegar-amended plants than in control plants. Related to the previous statement on the role of root plaques in metal mobility, it is suggested that wood vinegar may have a positive effect on these substances which favor their activity. The most prevalent root plaque under wetland conditions is iron oxyhydroxide or commonly known as Fe plaques. The formation proceeds from the reduction of ferric ions (Fe^{3+}) to a more soluble ferrous ions (Fe^{2+}) then oxidation resulting in iron oxyhydroxide plaques (FeOOH). One of the factors that affect the formation is the release of phenolic compounds by the

Table 6. Bioconcentration (BCF) and translocation factors (TF) of heavy metals as affected by wood vinegar application in contaminated soil planted with rice

Treatment/Variable	BCF	TF (straw)	TF (grains)
	Copper		
Control	0.06 c	0.00 b	0.32 a
CS	0.09 b	0.14 a	0.21 b
CS + 100-x WV	0.09 ab	0.13 a	0.17 b
CS + 250-x WV	0.09 b	0.14 a	0.18 b
CS + 500-x WV	0.10 a	0.15 a	0.17 b
Lead			
Control	0.06 b	0.36 a	0.00 c
CS	0.06 b	0.32 a	0.12 a
CS + 100-x WV	0.08 a	0.18 a	0.09 ab
CS + 250-x WV	0.06 b	0.23 a	0.08 b
CS + 500-x WV	0.09 a	0.27 a	0.09 ab
Zinc			
Control	0.10 c	0.79 a	0.44 a
CS	0.11 c	0.37 b	0.30 b
CS + 100-x WV	0.11 c	0.35 b	0.33 b
CS + 250-x WV	0.19 b	0.23 c	0.16 c
CS + 500-x WV	0.22 a	0.16 c	0.14 c

Note: Values within a parameter followed by the same letter(s) are not significantly different among treatments at 5% level based on Tukey's HSD test.

plant's roots with decreasing pH (Mendelssohn et al. 1995). In this study, the application of wood vinegar considerably decreased soil pH. Moreover, the phenolic compounds contained in the wood vinegar might have contributed to the reduction process of Fe^{3+} to Fe^{2+} thereby increasing substrate for plaque formation.

The plant's permissible limits for Cd, Cu, Pb, and Zn recommended by WHO are 0.02, 10.0, 2.0 (Hassan et al., 2012), and 50.0 mg kg^{-1} (Shah et al., 2011), respectively. All treatments did not show traces of Cd in grains, straw, and roots of rice. Although Cu, Pb, and Zn were present in all parts of the plant, the values obtained in the edible part were below the limits for possible contamination. The concentration of these metals in rice straw was also below the plant's permissible limits. The concentration of Pb and Zn in roots was increased after wood vinegar application. Lead concentration in roots was above the allowable limit with increased values after application of wood vinegar. Although Pb was observed to be higher than the acceptable values, the Pb concentration in roots was far below the concentration in soil which indicates that rice does not have the ability to accumulate Pb (together with Cu and Zn) in their tissues therefore do not pose risk of metal contamination.

The amount of metals absorbed by the plants are controlled by the factors associated with: (1) the concentrations and speciation of the metal in the soil solution, (2) the movement of the metal ion from the bulk soil to the root surface, (3) the transport of the metal from the root surface into the root, and (4) its translocation from the root to the shoot (Alloway, 1995). It was observed in the experiment that the order of metal uptake in rice is the same as the order of metal concentration in soil: Cu < Pb < Zn. The high uptake of Zn can also be explained by its bioconcentration and translocation factors. Among the three metals analyzed, Zn has the highest BCF and TF in straw and grains. High values mean the most readily taken up and translocated of all the metals considered. (Alloway, 1995). In addition, Zn is a mobile element compared to Cu and Pb. Zinc having the highest concentration and its presence in the exchangeable fraction that can be readily absorbed by the plant contributes to the increase in the metal concentration in plants.

After absorption of metals through the roots or leaves and transport to xylem vessels, the

Table 7. Effect of wood vinegar application on heavy metal uptake in grains, shoots, and roots of rice planted in contaminated soil

Treatment/Variable	Cd	Cu	Pb	Zn
	(mg plant^{-1})			
Grains				
Control	-	0.00 b	0.00 b	0.09 a
CS	-	0.02 a	0.01 a	0.10 a
CS + 100-x WV	-	0.01 ab	0.01 a	0.10 a
CS + 250-x WV	-	0.02 ab	0.01 a	0.09 a
CS + 500-x WV	-	0.01 b	0.01 a	0.09 a
Shoots				
Control	-	0.00 b	0.02 a	0.29 a
CS	-	0.02 a	0.06 a	0.21 ab
CS + 100-x WV	-	0.02 a	0.06 a	0.21 ab
CS + 250-x WV	-	0.02 a	0.06 a	0.20 ab
CS + 500-x WV	-	0.02 a	0.07 a	0.16 b
Roots				
Control	-	0.02 b	0.02 c	0.09 d
CS	-	0.04 a	0.05 b	0.15 c
CS + 100-x WV	-	0.03 a	0.06 ab	0.12 cd
CS + 250-x WV	-	0.04 a	0.08 a	0.26 b
CS + 500-x WV	-	0.04 a	0.07 a	0.32 a

Note: Values within a parameter followed by the same letter(s) are not significantly different at 5% level based on Tukey's HSD test.

possibility of movement through the whole plant is there. Several factors affect the rate and magnitude of movement within plants such as the plant organ, plant age, and metal concerned. As cited by Alloway (1995), Zn is readily translocated, Cu is intermediate, and Pb is least translocated to the aboveground plant parts. The degree to which metals are translocated after root absorption increases in the order Pb < Cu < Zn. These statements support the findings of the experiment in the uptake of metals from root to shoot then to grains. Zn has the highest uptake in grains followed by Cu then Pb.

Effect of wood vinegar on heavy metal availability and fractionation in soil

In the study, the pH in paddy soil had a noticeable decrease after the addition of heavy metal solution and wood vinegar but still in near neutral condition (Table 8). The most concentrated wood vinegar resulted in the lowest soil pH. Numerous studies (Kashem and Singh 2001; Antoniadis et al. 2008; Usman et al. 2008) showed that the adsorption and desorption characteristics of soil are associated with soil properties including pH, cation exchange capacity, redox reactions, organic matter content, composition of clay minerals, carbonates, Fe and Mn oxides. Because of its profound effects on metal solubility and speciation in soil, pH was regarded to have the most essential role in the determination of metal solubility, speciation, mobility, and bioavailability (Mühlbachová et al., 2005; Zhao et al., 2010). One of the major components of wood vinegar is acetic acid which is largely responsible for soil acidity. The phenolic compounds in wood vinegar somehow also contribute in pH decrease. The concentration of Cu, Pb, and Zn in all fractions analyzed was increased after a decrease in soil pH attributed to wood vinegar application (250-x). This agrees with the study cited by Zeng et al. (2011) which indicates an intense increase in the release of heavy metals, especially Pb and Zn from soil when pH decreased. Theoretically at pH values around 6 or 7, minimal amounts of metals are likely to be found in solutions. This can be exemplified by the availability of Zn in the exchangeable fraction after wood vinegar application. Although wood vinegar decreased soil

Table 8. Influence of wood vinegar application on pH of soil planted with rice in contaminated soil

Treatment/Variable	Soil pH
Control	7.63 a
CS	7.40 b
CS + 100-x WV	7.38 b
CS + 250-x WV	7.40 b
CS + 500-x WV	7.54 a

Note: Values within a parameter followed by the same letter are not significantly different at 5% level based on Tukey's HSD test.

pH, the concentrations used maybe not be that strong enough to create a large impact in lowering pH that will eventually affect the mobility/availability of Cu and Pb in the carbonate and exchangeable fraction.

The effect of WV on paddy soil is not straightforward having a dynamic soil system. Acidification is not reflected in the soil pH data because of a submerged soil condition. The pH that was considered is the bulk soil. There could be a unique effect of the WV when rhizosphere soil was only considered. There's a difference of redox condition between the rhizosphere soil and bulk soil. Although the rhizosphere soil is not analyzed separately, there would be a more profound effect on physicochemical characteristics of the soil such as pH, EC, and concentration of available elements (essential and non-essential). To fully elucidate the effect of WV application in rice soil system, this warrants a more detailed investigation on the dynamics of rice rhizosphere processes. Nonetheless, the result indicates that the soil pH is negatively correlated with the concentration of heavy metals present in the different soil fractions.

Total heavy metal content provides limited information on the availability and behavior of metals therefore is not sufficient in evaluating contamination (Adamo et al., 2002). Heavy metals are distributed in different soil fractions, which may strongly influence their behavior in soil. Hence, sequential extraction for speciation of heavy metals is necessary to determine their bioavailability, mobility, and possible toxicity. Metals in soil occur in various forms namely, exchangeable fraction wherein heavy metals are specifically adsorbed and are possible to be released depending on the ionic composition of water; carbonate fraction in which metals are precipitated or co-precipitated and are unstable under reducing conditions; Fe–Mn oxide bound fraction which is relatively stable under normal conditions; OM bound fraction which is also relatively stable wherein metals can be absorbed or form complexes. Under oxidizing conditions, OM can be degraded to result in the release of heavy metals. Last is the residual fraction wherein metals are contained in the crystal lattices of some primary and secondary minerals. Metals associated with this fraction are least labile and unlikely to be released under normal conditions (Zhao et al., 2010).

Five fractions were analyzed in this study based on the sequential extraction scheme which corresponds to the various metal forms in soil. Presented in Figures 1, 2, and 3 is the concentration of Cu, Pb and Zn in the different soil fractions. Addition of heavy metal solutions in soil resulted to an increase in Cu, Pb, and Zn concentrations. However, application of wood vinegar (500-x dilution) generally decreased the concentration of the three metals in all fractions.

Copper was not detected in the exchangeable and carbonate bound fractions. Regardless of treatments, Cu concentration was highest in the residual fraction (Figure 1c). Wood vinegar with the lowest concentration significantly decreased the concentration of Cu in Fe-Mn oxide bound, OM-bound, and residual fractions (Figure 1a, 1b, and 1c). The decrease in concentration were 29.78%, 30.87%, and 11.83%, respectively. Similar to Cu, no traces of Pb were detected in the exchangeable fraction. However, bulk portion of Pb was found to be in the Fe-Mn oxide fraction regardless of treatment applications (Figure 2b). The concentrations of Pb in the remaining fractions were reduced by wood vinegar application except for 250-x dilution (Figures 2a, 2b, 2c, and 2d). This treatment significantly increased the Pb concentration in the four fractions by 29.21%, 33.70%, 26.04%, and 20.33% respectively. The majority of Zn was bound to the residual fraction (Figure 3e) as observed also in Cu. A significant reduction in Zn concentration was seen in the exchangeable (9.47%), OM bound (12.60%), and residual fraction (4.35%) when wood vinegar was applied at 500-x dilution (Figures 3a, 3d, and 3e). Although insignificant, Zn bound to Fe-Mn oxides was also reduced by this treatment (Figure 3c).

The highest concentration of Cu and Zn was found in the residual fractions which are in insoluble forms therefore unavailable for plant uptake. This can also indicate the degree of environmental pollution. The higher the concentration of metals in this fraction, the lower the degree of pollution.

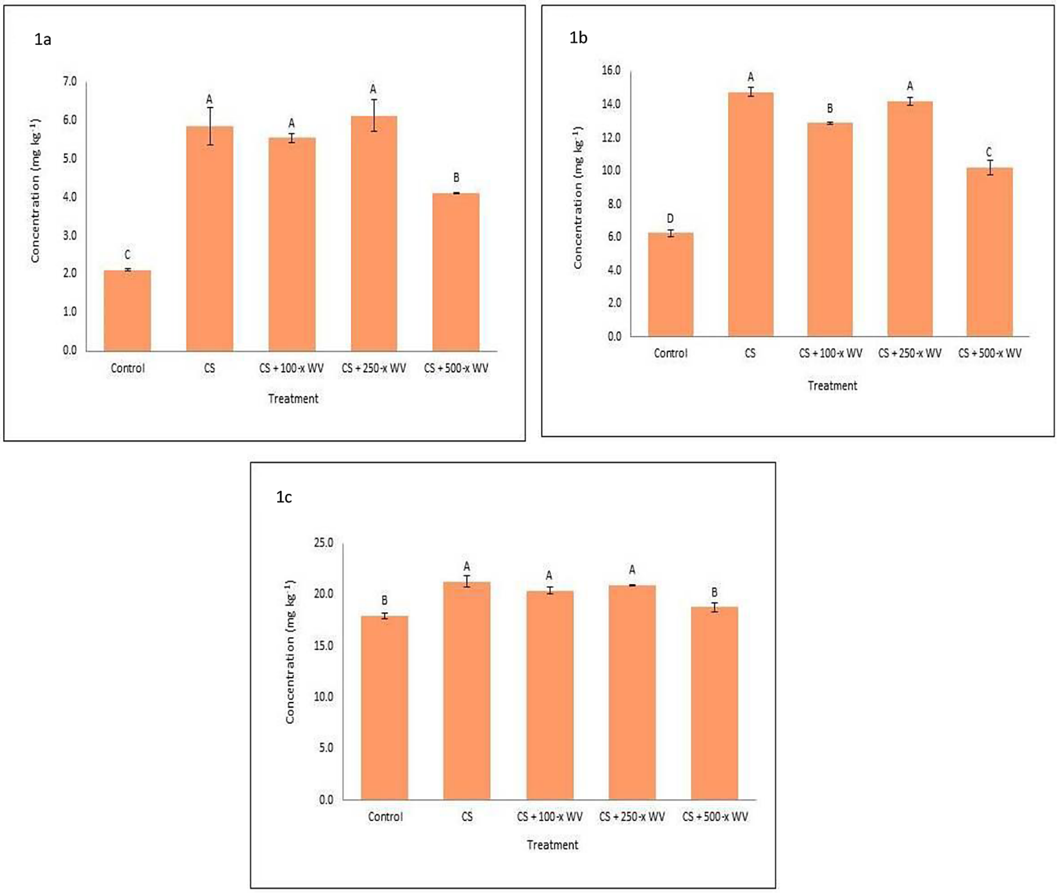

Figure 1. Copper concentration in different soil fractions as influenced by wood vinegar application in contaminated soil planted with rice: bound to Fe-Mn oxides (a), bound to organic matter (b), residual fraction (c), values are the means ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) between the treatments. Vertical bars represent standard error

The higher percentage of Pb in the Fe-Mn oxide fraction than the residual fraction that somehow was brought about by the application of wood vinegar and relatively high percentage of Zn also in the Fe-Mn oxide fraction have the tendency to be mobilized under reducing and acidic conditions.

The strong affinity of low-molecular-weight organic acids with heavy metals has regarded these compounds to be well-known in enhancing the mobility and remediation efficiency for metals. The formation of complexes between metals and organic acids will result in soluble complexes that will prevent metals from being adsorbed or precipitated. The presence of organic acids (acetic acid, phenol, and propanoic acid) in wood vinegar serves as suitable reactive sites for metal complexation. Possibilities of increased metal in soil solution due to enhanced metal solubility or decreased metal concentration in soil due to plant uptake or movement in a more stable fraction can occur. Based on the result of the experiment, it is suggested that the lowest values of Zn obtained in the exchangeable fraction was due to uptake by the rice plant. However, the non-detection of Cu and Pb in the exchangeable fraction was due to the stronger affinity of these metals in the carbonate and OM fractions. Although Cu and Pb are considered as immobile elements, their presence in plants indicates bioavailability of these metals. The application of wood vinegar has a profound effect rendering these metals available for uptake. The stability constant of Cu is higher than Pb and Zn which makes it more soluble or available for plant uptake (Chen and Stevenson, 1986). The decrease in heavy metal concentration observed in soil after wood vinegar application can also be due to the redistribution in other fractions or change of metal forms into more available forms resulting to plant uptake. Berkelaar and Hale (2003) recommended that the metal complexes contribute to uptake of metal by plants through direct uptake of the complexes or by lessening

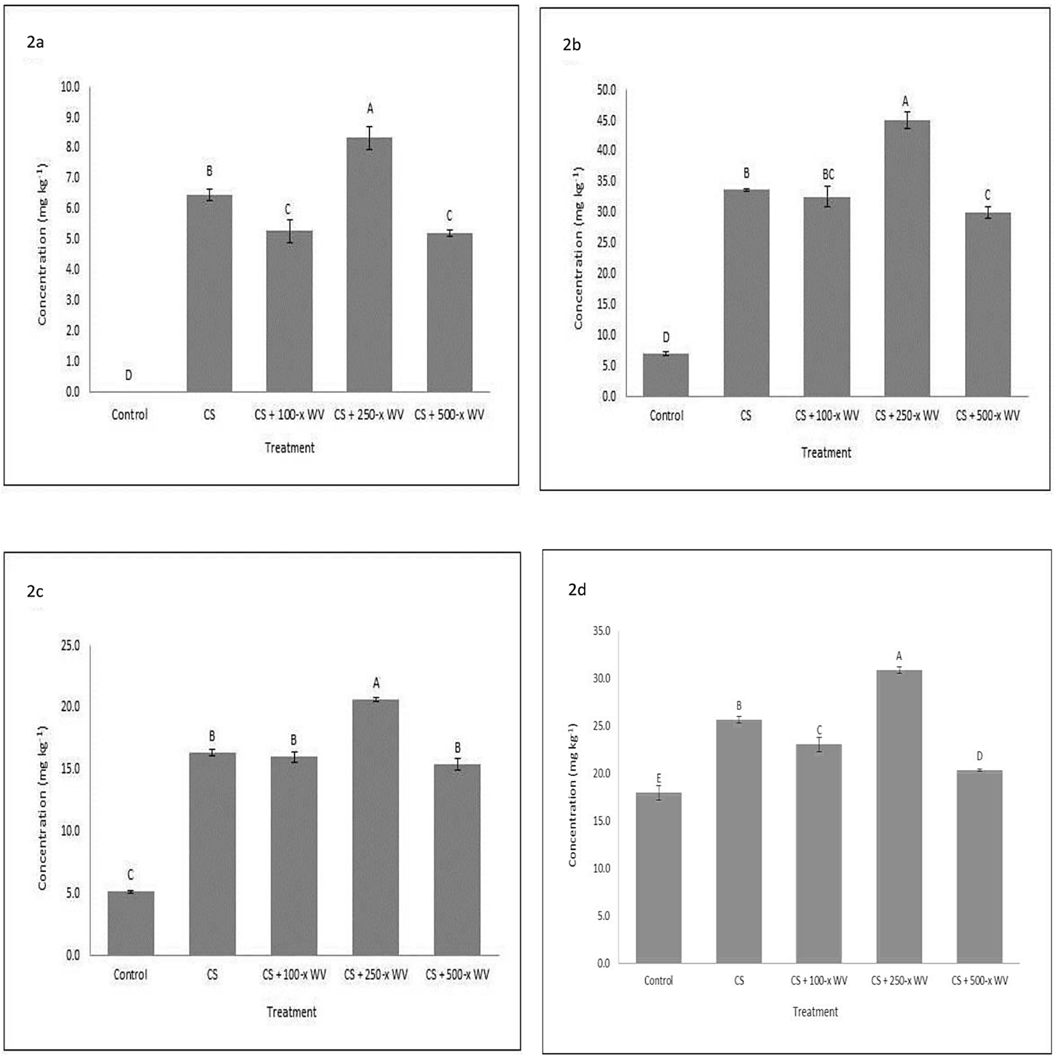

Figure 2. Concentration of lead in different soil fractions as affected by wood vinegar application in contaminated soil grown with rice: bound to carbonates (a), bound to Fe-Mn oxides (b), bound to organic matter (c), residual fraction (d), values are the means ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) between the treatments. Vertical bars represent standard error

diffusional constraints for uptake of free ions such as dissociation of labile metal complexes.

The presence of ammonia in wood vinegar also influences the availability of Cu in solution. The reaction of Cu with water results in hydrated Cu^{2+} ions $[Cu(H_2O)_6]^{2+}$ which is soluble in water. Available OH- ions from ammonia in solution will react with the hydrated Cu ion producing an insoluble $Cu(OH)_2$. However, if more ammonia solution is added, the insoluble form will be dissolved forming the soluble complex ion: $[Cu(NH_3)_4]^{2+}$. Based on soil pH, the dominant Cu species in solution above pH 7.0 is $Cu(OH)_2$. All pH values obtained from the experiments are above 7.0 which indicates that the bulk of Cu is in insoluble form. This is also reflected in the fractionation of Cu in soils. Copper is specifically adsorbed or fixed in soil making it more immobile. But as mentioned earlier, the reaction between the insoluble Cu and ammonia in solution will render these species available. The concentration of the available form of Cu in the soil might either be low enough or readily taken up by the plants resulting to non-detection in soil and low uptake in plants.

General observations showed a decrease in percentage of Cu, Pb, and Zn in CS treated soil in the residual fraction while a percentage increase in the remaining fractions in the same treatment (Figures 4a, 4b, and 4c). Regardless of treatment applications, it was observed that Cu has the highest percentage in the OM fraction. This is because Cu has a high capacity to complex with OM. Moreover, Bolan et al. (2003) stated

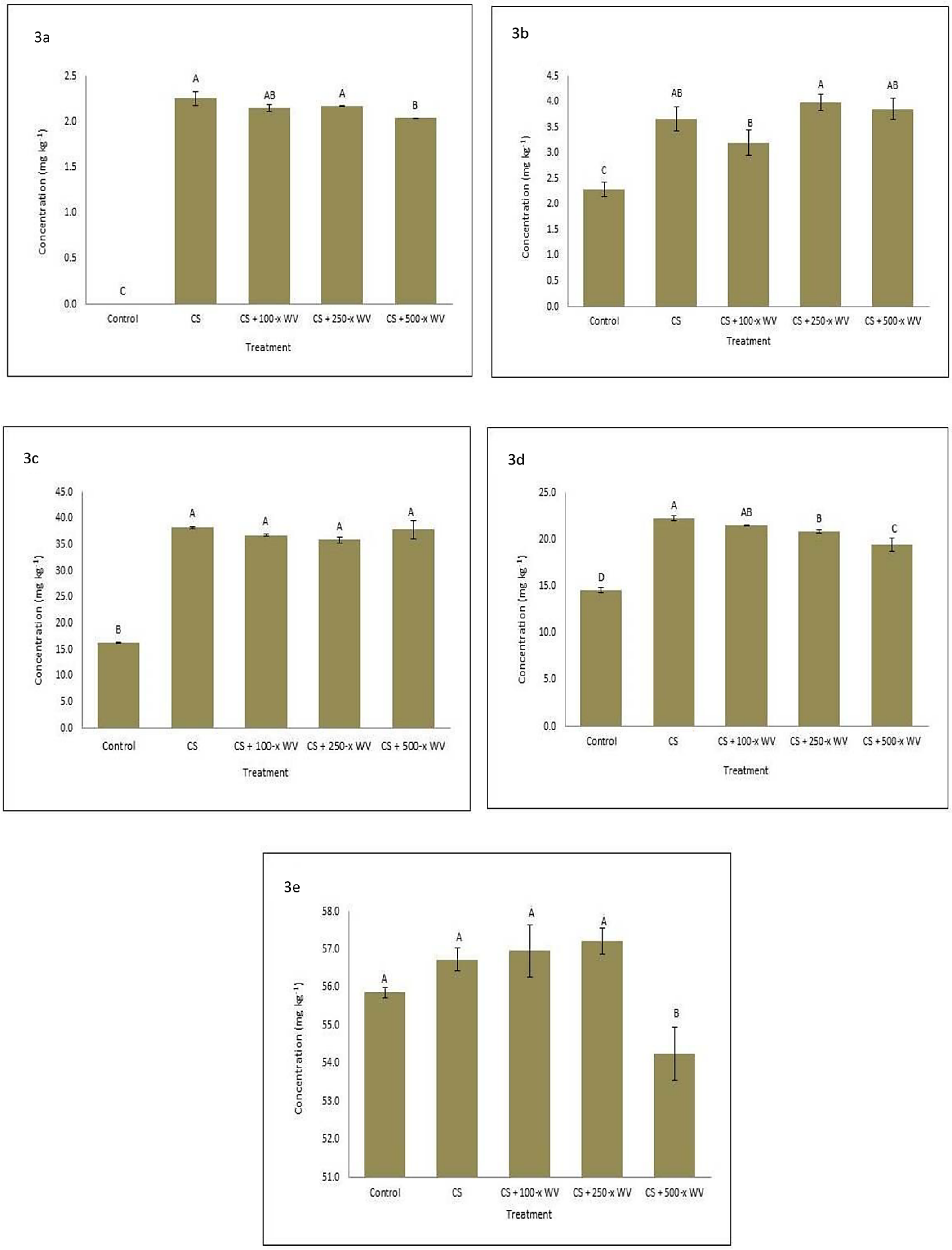

Figure 3. Zinc concentration in different soil fractions as affected by wood vinegar application in contaminated soil grown with rice: exchangeable fraction (a), bound to carbonates (b), bound to Fe-Mn oxides (c), bound to organic matter (d), residual fraction (e). Values are the means ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) between the treatments. Vertical bars represent standard error

that the stability constant of Cu with humic acid is higher compared to the stability constants of other heavy metals. Similar study in paddy soils showed that greater than 50% of the total Cu was associated in the OM fraction which suggests strong relationship between Cu and organic matter (Khairiah, 2012). Based on positive control, a notable increase in percentage Cu was seen in the residual fraction when 100-x WV and 500-x WV were applied but a significant decrease was manifested in the OM-bound fractions. An opposite trend was found in Pb. The application of wood vinegar resulted in a percentage reduction in the residual fraction while a percentage increase was

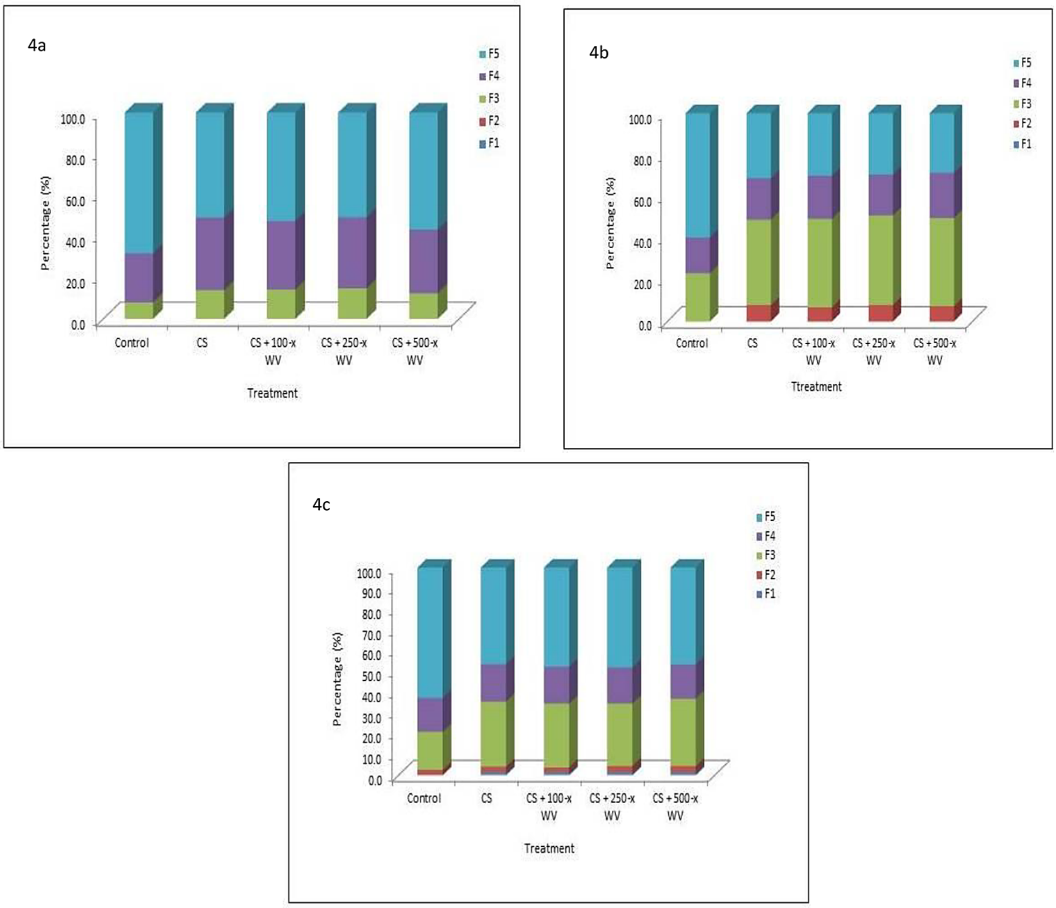

Figure 4. Percentage of copper (a), lead (b), and zinc (c) in each fraction as affected by the application of wood vinegar in contaminated soil planted with rice. F1 – exchangeable; F2 – bound to carbonates; F3 – bound to Fe-Mn oxides; F4 – bound to OM; F5 – residual

generally seen in the Fe-Mn oxide and OM bound fractions. Almost similar findings in Cu were exhibited by Zn. Wood vinegar application in soil also effected a percentage increase in Zn in the residual fraction. However, a common decrease in percentage of Zn was observed in other fractions. The various changes in the percentage of metals in each fraction indicate that wood vinegar can displace ions and enhance mobility in soil.

Correlation between heavy metals in rice and soil

Correlation analysis between heavy metals in rice and the different soil fractions showed that the Cu in grains, straw, and roots are positively correlated with the soil fractions – bound to Fe-Mn oxide, bound to OM, and residual fraction. The highest positive correlation was seen between Cu in roots and Cu bound to Fe-Mn oxides and OM ($r = 0.941$ and $r = 0.924$, respectively). The positive correlation of Cu and Pb in rice grains, straw, and roots with the Fe-Mn oxide and OM bound fractions indicates a possibility for translocation of the metal in the plant. This will happen provided with favorable conditions. The metal held in the Fe-Mn oxide fraction will be mobilized under acidic and reducing environment. Although the metals in the OM bound fraction are relatively stable, these can also be mobilized under strong oxidizing conditions due to OM degradation. Strong positive correlations were also observed between Pb in rice and the four soil fractions analyzed. Lead in carbonate fraction can be precipitated and unstable under reducing condition. Varied results were obtained in correlation analysis between Zn in plants and soil. A strong negative correlation, except for residual fraction, was seen between Zn in straw and the remaining fractions. An opposite of this was observed in Zn in roots. A positive correlation in all soil fractions except for the residual fraction was seen. With the positive correlation between Zn in roots and the available and reactive fractions, the application of a more diluted wood vinegar which resulted in a decrease in Zn in all fractions could also possibly lower the Zn uptake in roots which will eventually decrease metal concentration in the plant.

CONCLUSIONS

It is indicative of the results that rice biomass and yield benefitted from the application of wood vinegar at a lower concentration (500-x). Wood vinegar at high concentration can be toxic to plants, due to the chemical components present. These include acetic acid, phenols, and other organic acids. At high concentrations, these substances can disrupt plant growth and cause physiological problems. In addition, increasing concentration of WV could result in temporary acidification of the soil that eventually result to nutrient imbalance. The results showed an inverse relationship between the concentration and the effect of WV implicating a toxic effect at higher concentrations.

Results also revealed that the uptake of heavy metals was reduced by wood vinegar application and found to be below the permissible limits in plants. Rice field has a complex environment with defined flooded and non-flooded conditions which makes the behavior of metals more complicated. Cu, Pb, and Zn bound in the residual fraction do not pose a risk in soil or plant contamination. However, those associated with relatively stable fractions (carbonate-, Fe-Mn oxide- and OM-bound) have the tendency to become bioavailable provided with favorable conditions. The increase in Cu and Pb concentrations after applying wood vinegar at 250-x should be taken into consideration. Variations in percentage of metals in each fraction indicate that wood vinegar is capable of incurring changes in metal distribution in soil through displacement or mobilization thereby affecting the concentration and uptake by plants. However, the specific effect of each of the WV component such as phenols, organic acids, and ammonia on the available fractions or metal-organic complexes were beyond the scope of this study which warrants further research to elucidate their effects.

It is recommended to apply 1.600 cubic meters of 0.2% WV in a one-hectare field to ameliorate the effects of heavy metals in the soil. Application can be done simultaneously with irrigation schedules to minimize labor costs, but further investigation is warranted to fully understand the long-term effects of WV applications under field conditions.

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