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Combined effects of water retaining agent and green manure on trace elements concentration in tobacco farming purple soils

Angelique Iradukunda[1,2], Dan Zhang[1]*, Tianhui Ye[3], Ernest Uwiringiyimana[1,2,4], Lu Xu[1] and Solange Uwamahoro[2,5]

[1]Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu 610041, China.
[2]University of Chinese Academy of Sciences, 100049, China.
[3]Panzhihua Branch of Sichuan Tobacco Company, Miyi County Government Affairs Center, 617299, China
[4]Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China.
[5]State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China.

Green manure (GM) is widely adopted for agricultural productivity and sustainability. The present study supplemented the GM effect with that of water retaining agent (WRA) to enhance the sustainability of tobacco planting purple soils in Sichuan Province, China. The study employed three GM legumes varieties (*Trifolium repens, Vicia villosa* and *Medicago sativa*), while the WRA used was polyacrylamide at different application rates (0, 30, 90, 150 and 210 kg/ha). The results revealed that the average concentration of trace elements ranged from 0.86 to 1.18 mg/kg, 22.2 to 26.5 g/kg, 78 to 426 mg/kg, 16 to19 mg/kg, and 69 to 76 mg/kg for Mo, Fe, Mn, Cu, and Zn, respectively. Compared to actual soil concentration, Mn was increased by adding 150 or 210 kg/ha to *M. sativa* fertilized soil. Zn was increased by combining any WRA rate with *V. villosa* or 150 kg/ha WRA with *T. repens*. The combination of 90 kg/ha WRA with *M. sativa*, and 30 kg/ha WRA with either *T. repens* or *V. villosa* decreased Fe. Then, all the treatments reduced soil Mo and Cu. Therefore, these GM and WRA affected the soil environmental conditions associated with different responses of individual soil trace elements.

Key words: Green manure, water retaining agent, purple soil, soil trace elements.

INTRODUCTION

Green manure is an agricultural product created after leaving plants fresh materials either in place or brought from afar to wither on field surfaces so that they can be used as mulch and/or soil amendment later (Prado Wildner et al., 2004). The green manuring practice started in the ancient time and gained much popularity in

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the first decades of the 20th century (MacRae and Mehuys, 1985), but received considerable attention from the research community in recent decades (McCauley, 2011; Miller et al., 2011; Lee et al., 2010; Eilittä et al., 2004; Hartwig and Ammon, 2002). Green manuring is mainly related to organic farming, hence plays a vital role in sustainable agriculture, mostly in annual cropping systems (Sumiahadi et al., 2020; Eilittä et al., 2004). *Medicago sativa* L. is a versatile leguminous crop that has long been known as a soil remedy (Huhman and Sumner, 2002; Peralta et al., 2001). The most remarkable performance of *Trifolium repens* L. on nitrogen (N) production was also reported earlier by Barney (1987). On the other hand, Asagi and Ueno (2009) found that *T. repens* L. and *Vicia villosa* Roth were the most efficient N sources for rice yield. It was previously reported that the benefits associated with weed control, water holding capacity, rhizosphere soil ecology, and soil conservation might be attributed to *V. villosa* (Dong et al., 2021; Choi and Daimon, 2008; Hartwig and Ammon, 2002). Thus, the green manure improves soil conditions, nutrient availability and cycling, and soil physical properties (Ma et al., 2021; Karkanis et al., 2007). Although several GM varieties have been used in previous studies (Ma et al., 2021; Bilalis et al., 2009; Karkanis et al., 2007), the purposes of the studies were limited to one crop variety or macronutrients.

Karkanis et al. (2007) recommended the combination of green manure and irrigation application in the field to improve tobacco productivity. It has been shown that the supply of water in the rhizosphere influenced the increase in the productivity of tobacco (Bilalis et al., 2009). Previous studies revealed that water-absorbing soil amendment might improve soil structure, raise water storage, increase irrigation water use efficiency, and decrease other water-related limitations (Salim, 2015; Maghchiche et al., 2010; Huettermann et al., 2009). WRA also reduces soil nutrients loss (Roshanizarmehri et al., 2018; Xuefeng et al., 2017; Kim et al., 2015; Jiang et al., 2010); thus, WRA application on arable land is a technique that may facilitate in growing healthy crops such as tobacco and enhances good quality harvest. Polyacrylamide is a synthetic hydrogel polymer that has considerably shown its effectiveness in improving soil physical condition and water retention capacity among other WRA (Maghchiche et al., 2010; Green and Stott, 2001; Seybold, 1994). Molybdenum (Mo), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) are the focus of this study because the tobacco plant requires them for better growth and good quality of leaves. Generally, Zn plays its primary role in synthesizing proteins, enzyme activation, oxidation and revival reactions, and metabolism of carbohydrates in plants (Mousavi et al., 2013). Cu acts as an essential cofactor of numerous proteins, with the cell wall as one of the significant Cu-accumulation sites in plants (Printz et al.,

2016). On the other hand, molybdo-enzymes are also involved in synthesizing the phytohormones (Kaiser et al., 2005). Although all the aforementioned elements are micronutrients, their deficiency negatively affects the tobacco leaves, which are valuable parts of the tobacco plant because they are used to make cigarettes in different parts of the world.

Purple soils of China are valuable agricultural soils that resulted from weathering of sedimentary rocks of purple color (He et al., 2009). These soils can be acidic, neutral, or alkaline (Xiao et al., 2016); and their mineral nutrient contents with the co-occurrence of abundant rainfall and a warm climate make the area of purple soils a favorable environment for crop growth (Zhong et al., 2019). Purple soils of Sichuan (China) are typically used to grow tobacco, which is one of the main crops in that area (Xu et al., 2021). However, a recent report showed that the concentration of organic matter content is generally low in purple soils of Sichuan region (Zhong et al., 2019) and is a critical limiting factor in agricultural production. Specifically, the decline in soil organic matter content leads to soil fertility loss (Lin et al., 2007). According to Zou et al. (2008), micronutrients deficiency in crops of China is due to the concentration of soil trace elements, which has an average of 100 mg/kg, 2.94%, 710, 1.7, and 22 mg/kg for Zn, Fe, Mn, Mo, and Cu, respectively. To cope with the soil fertility problem, tobacco growers use synthetic fertilizers, pesticides, and growth regulators (Lecours et al., 2012). However, it has been projected that further use of these synthetic fertilizers at a higher rate might cause substantial negative impacts on the environment (Ren et al., 2017). Long-term tobacco plantation has induced soil acidification with 1.93 kmol H^+/ha/year production, mainly derived from chemical fertilizer input and plant N uptake in purple soils (Zhang et al., 2016). In addition, soil acidification can cause the deficiency of some soil mineral elements. The acidifying effects of tobacco cultivation and soil nutrients depletion were studied by Moula et al. (2018), who found that the average values of inherent chemical properties decreased substantially after two consecutive years.

As the need for a sustainable technique in agronomy which is environmentally friendly is still a necessity for farmers and environmentalists, the present study used GM and polyacrylamide as WRA, to enhance the sustainability and productivity of tobacco farming purple soils. This study aimed to assess the combined effect of GM varieties and WRA on soil trace elements and hypothesized that this could possibly improve the level of trace elements in the soil of the studied area.

MATERIALS AND METHODS

Study area description

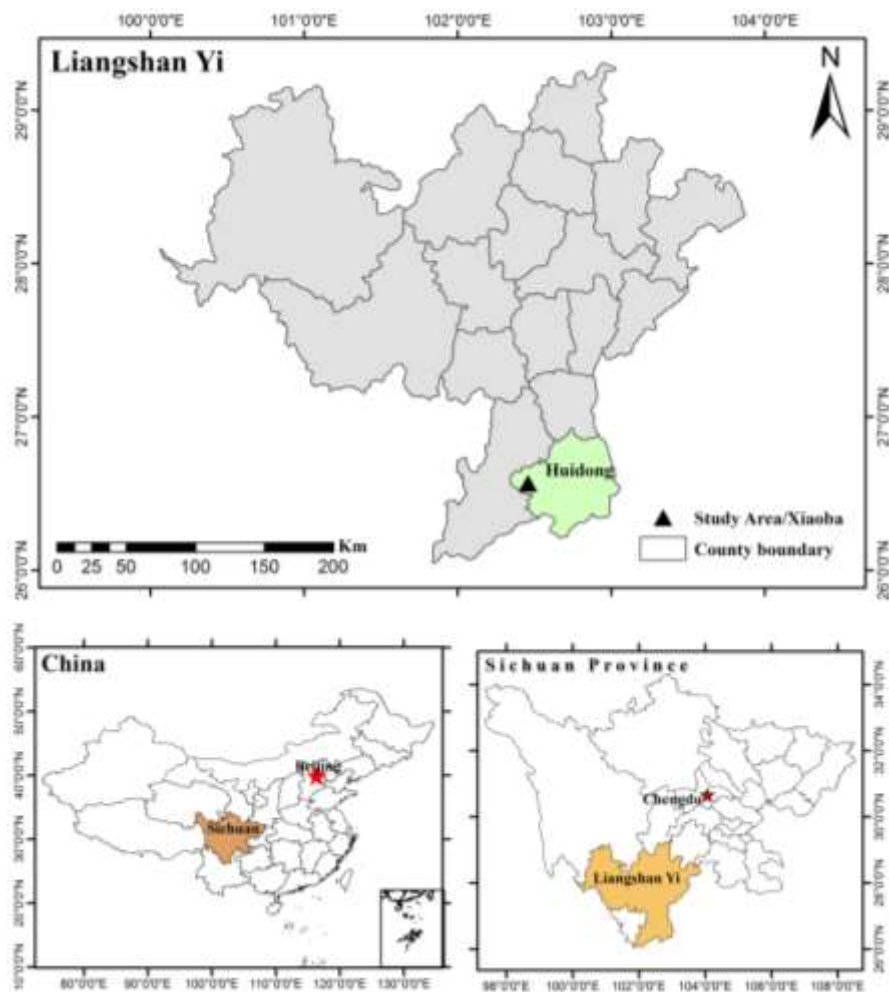The study was established in Xiaoba township, Huidong County,

Figure 1. Geographical position of the study area.
Source: Authors (using ArcMap version 10.4 software (https://desktop.arcgis.com/))

Sichuan Province in China. Huidong is a mountainous agricultural county, under the authority of Liangshan Yi autonomous Prefecture (Figure 1). It is located on the Prefecture's Southern end and the Northwestern edge of the Guizhou-Yunnan Plateau and covers an area of 3227 km^2 with 132 inhabitants/km^2 as of 2018. Huidong has been honored to be the county with outstanding achievements in the development of modern tobacco agriculture. Due to its high topography (640 - 3330 m above sea level), the total runoff is estimated to be 1.498 billion m^3 each year. Huidong county experiences a humid monsoon climate zone in the western central subtropical zone. The high fluctuation of the rise and fall of the temperature in Huidong influences the average temperature for many years to be 16.2°C, while the average annual precipitation is 1095.5 mm. Our experimental study was conducted on purple soil; its properties were determined from the sample collected before field preparation. The properties of soil in the study area are: pH: 7.5; Total nitrogen (TN) in %: 0.032; Soil Organic Matter (SOM) in %: 0.421; Total Phosphorus (TP) in mg/kg: 271.5; Available K (AK) in mg/kg: 150.5; Ca in mg/kg: 1080.5; Mg in mg/kg: 1128.5; Mo in mg/kg: 1.25; Fe in g/kg: 22.84; Mn in mg/kg: 402; Cu in mg/kg: 21.7; and Zn in mg/kg: 71. The variety of tobacco called *Nicotiana tabacum L* is mostly cultivated in the study area and contributes to

the agricultural production of the county and the country in general.

Experimental design

The experiment was a year-round trial completed by applying GM legumes, and WRA called polyacrylamide on different plots in the study area. The plots were laid out in full factorial design of three GM varieties (*M. sativa, V. villosa,* and *T. repens*) and five application rates of WRA (0, 30, 90, 150, and 210 kg/ha) replicated thrice. The combination of one GM variety with one rate of WRA was considered as a single treatment. GM seeds per plot were 30 kg/ha for *T. repens*, 45 kg/ha for *M. sativa*, and 45 kg/ha for *V. villosa*.

Agronomic practices and sample collection

Before GM and WRA application, the soil was loosened and ditches were levelled. The application of treatment started with the sowing of GM in the winter time. The slope of each plot was 15°. GM seeds were drilled in furrows spaced out by 30 cm and bury them with soil.

Figure 2. Flowering of the three GM varieties ready to be incorporated in soil.
Source: Authors

Whereas WRA was applied using the broadcasting method. Then, the plots were watered for the first time to provide the optimum conditions for faster germination and WRA dissolution. At the flowering period of the GM varieties (Figure 2), these legumes were incorporated into their corresponding soil that they were grown on. The period from sowing to flowering was estimated to be five to six months, depending on the time taken by each variety to blossom. After incorporating GM in the soil, the undisturbed soil was left over few weeks before tobacco transplantation, while biogeochemical processes took place. Then after those processes, soil samples were taken from each treatment.

During the soil sampling, each plot was divided into four subparts, and each soil sample within the subpart of a given plot was obtained from the top 30 cm, usually considered as the root zone in cultivated soil (Franco-Hermida et al., 2017), by employing a zigzag pattern (Carter and Gregorich, 2007). With the aid of a soil Auger, soils samples from each plot were taken. Thereafter, one composite sample was made for each plot to represent one single treatment. Then the date and field details constituting a soil sample were used as the label on the plastic bag of the samples, and the labelled samples were transported to the soil laboratory for trace element determination and analysis.

Sample treatment in laboratory and analytical analysis of trace elements

Crucibles were washed using tap water, then soaked in 10% HNO_3 solution for 12 h, and finally, they were washed with deionized water and dried in a hot air oven for 1 h 30 min. Then, for each treatment, 0.15 g of ground air-dried sample was taken from its package using a spatula then weighed using an electronic balance. The weighed 0.15 g was placed in a crucible, and the label used corresponds to the one put on the packaging to represent the treatment used in the field experiment. Then, samples were digested using 65% HNO_3, 70% $HClO_4$, and HF; inductive coupled plasma-mass spectrometry (ICP-MS) was used to determine the total recoverable trace elements (Melaku et al., 2005).

Data analysis

The data were subjected to SPSS software and the analysis of variance (ANOVA) was performed. The mean significant differences among GM varieties with different WRA rates were determined by Tukey Honest Significant Difference Test at 5% of significance level. Then graphs were plotted using Origin software.

RESULTS AND DISCUSSION

The two factors considered in this study, GM and WRA, significantly influenced the concentration of trace elements (Mo, Fe, Mn, Cu, and Zn) in purple soils.

The combination of WRA and GM on purple soil showed the highest mean value of Mo (1.18±0.13 mg/kg) in *T. repens* amended soil under 90 kg/ha of WRA rate (Table 1). On the other hand, the lowest concentration of Mo (0.86±0.08 mg/kg) was from plots fertilized with *T. repens* without application WRA (Table 1).

The data of soil Fe concentration showed the lowest mean value (22.22±0.44 g/kg) in plots amended by *T. repens* with 30 kg/ha of WRA and the highest mean value (26.5±0.42 g/kg) in plots amended by *T. repens* without application of WRA (Table 1).

The mean values of Mn concentration recorded in the soil from each plot ranged from 78±7.5 to 426±11.7 mg/kg, whereby, the lowest mean value of Mn detected in plots fertilized by *T. repens* without application of WRA and the highest mean value detected from the plots fertilized by *M. sativa* with 150 kg/ha of WRA (Table 1).

In the present study, the soil Cu concentration from each plot ranged from (16±0.5 to 19±0.4 mg/kg). The lowest mean value of Cu was recorded in plots that received *Trifolium repens* with no application of WRA, while the highest mean value of Cu recorded in soils from the plots fertilized by *M. sativa* without application of WRA (Table 1).

The quantity of soil Zn for all treatments ranged between 59±0.5 mg/kg detected in plots amended by

Table 1. Concentration of soil trace elements from interaction effect of WRA and GM.

WRA (kg/ha)	Mo (mg/kg)	Fe (g/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Trifolium repens					
0	0.86±0.09[b]	26.50±0.42[a]	78±7.5[d]	16±0.5[c]	65±0.5[c]
30	0.99±0.07[ab]	22.22±0.44[c]	99±6.6[c]	16±0.9[c]	64±0.5[c]
90	1.18±0.13[a]	23.23±0.45[b]	137±4.4[b]	17±0.7[bc]	68±0.2[b]
150	1.13±0.05[a]	23.86±0.13[b]	186±5.6[a]	18±0.8[ab]	73±1.9[a]
210	1.09±0.03[a]	23.96±0.06[b]	194±1.01[a]	19±0.4[a]	75±1.07[a]1[a]
Vicia villosa					
0	1.06807±0.06[a]	24.33±0.16[ab]	225±8.1[c]	18±0.9[a]	73±3.8[a]
30	1.08±0.10[a]	22.76±0.18[d]	245±12.3[bc]	18±0.6[a]	74±0.4[a]
90	1.03±0.04[a]	23.64±0.11[c]	257±6.5[b]	19±0.2[a]	72±1.4[a]
150	0.97±0.08[a]	24.34±0.85[a]	340±4.5[a]	18±0.5[a]	76±2.1[a]
210	1.03±0.04[a]	23.86±0.29[bc]	321±4.87[a]	18±0.6[a]	71±0.6[a]
Medicago sativa					
0	0.9±0.05[a]	24.54±0.23[a]	346±4.1[b]	19±0.4[a]	67±0.5[a]
30	0.89±0.05[a]	24.17±0.21[ab]	290±2.1[d]	18±0.5[a]	67±1.7[a]
90	0.92±0.05[a]	22.64±0.24[c]	312±6.3[c]	16±0.8[b]	61±1.0[b]
150	0.92±0.03[a]	24.07±0.14[ab]	426±11.7[a]	19±0.1[a]	59±0.5[b]
210	1.01±0.06[a]	23.76±0.48[b]	412±3.5[a]	18±0.1[a]	66±1.7[a]

The mean ± standard deviation for each element (Mo, Fe, Mn, Cu, and Zn) in soil, resulted from the application of green manure (GM) and water retaining agent (WRA) combination on the purple soil of the study area. 0, 30, 90, 150, and 210 kg/ha are rates of WRA in GM variety amended plot. The values of one element along the column (irrespective of the GM varieties) that do not have any similar small letter, are significantly different at 5% according to Tukey Honest Significant Difference.
Source: Authors

M. sativa with 150 kg/ha of WRA, and 76±2.1 mg/kg observed in plots amended by *V. villosa* with applied WRA rate of 150 kg/ha (Table 1).

Different plants have different abilities in nutrients uptake, and the use of different rates of WRA had a significant effect on soil trace elements in the present study. In addition, the decomposition of different legume plants after incorporation may release different quantities of organic matter into the soil, which would lead to different changes in a soil environment. It is important to note that the concentration of trace elements in purple soils differed by treatments, though, in some cases, the difference was not statistically significant. GM has been proved to significantly change the soil properties, although the specific parts of plants were not assigned to their specific contribution to soil health (Crotty et al., 2015). Crotty et al. (2015) found that different legumes influence the soil faunal diversity differently. It turns out that clover plants referred to as *T. repens* in the present study may have stimulated a more significant number of soil faunal diversity responsible for rapid mineralization of soil organic matter and release of mineral nutrients. In their study, Yang et al. (2018) suggested that planting hairy vetch in winter fallow increases nutrients of purple

soils due to the mobilization of root exudates and different soil types. In addition, the soil organic matter increased after incorporating GM into the soil (Adekiya et al., 2019). A difference in GM performance was observed (Grüter et al., 2017); where clover (*Trifolium alexandrinum* L.) showed better effects on soil mineral N concentration than other GM varieties studied.

Different quantities of WRA broadcasted in the field at the GM sowing time influenced the concentration of trace elements in tobacco farming purple soil. The statistically significant difference among different rates of WRA influence on trace elements in GM amended soils is attributed to the influence of WRA soil water content and other soil physical properties. As observed by Green et al. (2004), polyacrylamide effectively enhanced the stability, which implies the resistance of soil aggregate mineral nutrients to environmental loss. In addition, soil water content influences different soil chemical and physical properties, affecting the properties of plants grown on that specific soil. The twist in irrigation water requirement resulted in different status of macronutrients, micronutrients, and other leaf parameters of Alfalfa (*M. sativa*) (Elfanssi et al., 2018). Thus, in line with the increase of micronutrients due to fertilization that
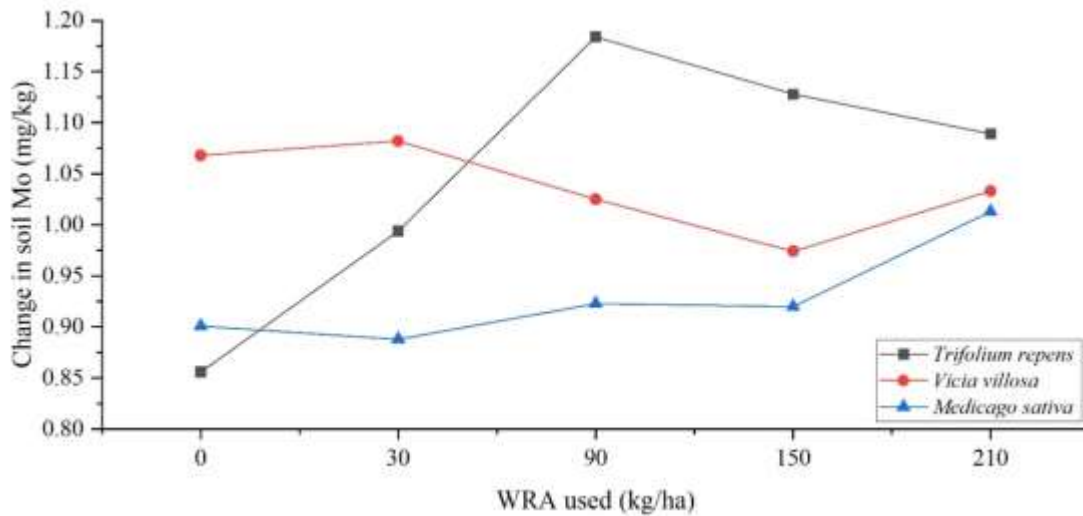
Figure 3. Levels of Mo in green manure and water retaining agent amended soil.
Source: Authors

provided an insight into the positive relationship between nutrients and the applied fertilizer (Lisuma et al., 2021), trace elements responded to the soil amendment.

Effect of green manure and water retaining agent on Mo

In plots amended with *T. repens,* the lowest mean value of Mo concentration (0.86 mg/kg) was observed when no WRA applied in soil (Figure 3), and the highest mean value of Mo content in soils (1.18 mg/kg) observed under 90 kg/ha of WRA (Figure 3). Moreover, these values were statistically different (p = 0.004) (Table 1). These results showed that the addition of WRA in the range of 30 to 210 kg/ha to the purple soil fertilized with *T. repens* resulted in the increase of Mo levels in the soils than solely using *T. repens* (Figure 3).

The difference between the highest levels of Mo (1.08 mg/kg) obtained under the application of 30 kg/ha WRA and the lowest level of Mo (0.97 mg/kg) obtained under the application of 150 kg/ha WRA in plots fertilized by *V. villosa* (Figure 3) was not statistically significant (p > 0.05) (Table 1).

For Mo concentration in soils from the plots that were amended with *M. sativa*, the plots treated with 30 kg/ha WRA recorded the lowest concentration of Mo (0.89 mg/kg), while the highest concentration of Mo (1.01 mg/kg) was recorded from the plots amended with 210 kg/ha of WRA (Figure 3). The mean difference was not statistically significant (p = 0.076) (Table 1). All levels of Mo observed under all treatments were lower than Mo concentration in the background properties of the study

area.

According to Fageria et al. (2002), Mo is the only trace element whose availability usually increases with soil pH rise. Moreover, Mo concentration in soil solution correlated with other soil parameters, including a positive correlation with pH (Rutkowska et al., 2017). On the other hand, the total Mo concentration decreases when Mo ions are withdrawn from their soil colloid to recover Mo taken by roots or leached out by environmental factors. In our experiment, GM varieties alone did not enhance the soil concentration of Mo. Although all WRA rates used in this study increased the level of Mo in plots fertilized with *T. repens* and *M. sativa*, the soil Mo concentration was still lower than in background properties.

Effect of green manure and water retaining agent on Fe

The mean difference between the highest and the lowest Fe levels in treated soil was statistically different (p < 0.001) (Table 1). In soils amended by *T. repens*, the lowest Fe concentration (22.22 g/kg) observed under application of 30 kg/ha WRA was lower than Fe concentration in background properties. In contrast, the highest Fe concentration (26.50 g/kg) observed without applying any rate of WRA (Figure 4) was higher than Fe content in background properties.

Besides, except for the lowest Fe concentration (22.76 g/kg) obtained under 30 kg/ha WRA, other concentrations of Fe in soils from the plots fertilized with *V. villosa* (Figure 4) were greater than Fe in background properties. As reported previously (Domagała-Świątkiewicz et al.,
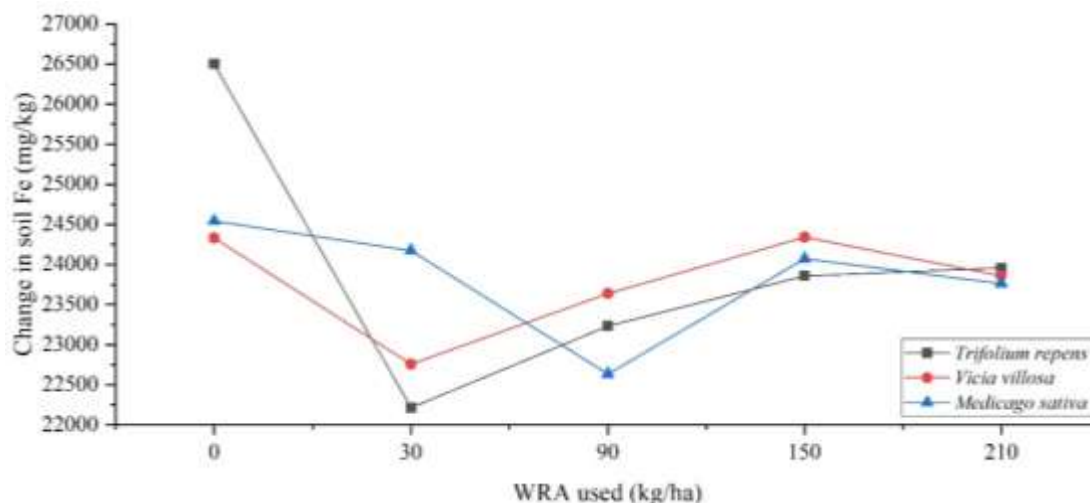
Figure 4. Levels of Fe in green manure and water retaining agent amended soil.
Source: Authors

2019), a higher concentration of Fe was observed in plots fertilized by *V. villosa* than in control plots in that study area.

Furthermore, the Fe content in soils collected from the plots amended with *M. sativa*, 90 kg/ha WRA resulted in the lowest concentrations of Fe in soils (22.64 g/kg) that was lower than Fe in background properties, while the highest concentration of Fe in soils (24.54 g/kg) observed without application of WRA in the soil was higher than Fe in background properties (Figure 4). Besides, all the plots fertilized with *M. sativa* with WRA resulted in lower levels of Fe concentration in soil than the plots without WRA amendment (Figure 4).

These results showed that the addition of WRA in the range of 30 to 210 kg/ha to the field fertilized with *T. repens* or *M. sativa* resulted in the reduction of Fe levels in the soils, as all plots amended with WRA exhibited lower Fe concentration than plots without WRA under those two GM varieties fertilization.

In the present study, GM increased Fe concentration in soil probably due to its ability to enhance soil micro bio-activity and its contribution to organic matter. As reported previously, Fe^{2+} content changed after the incorporation of green manures in soil (Gao et al., 2017). The noteworthy is that WRA application decreased the concentration of Fe in plots fertilized by GM. That may be attributed to soil factors (soil water conditions, redox status, the concentration of nutrients, and soil organic matter) that could influence the reduction of Fe^{3+} (Heiberg et al., 2012). Fe^{3+} reduction in paddy soil was affected by soil conditions and environmental factors, especially the soil water and redox ability; thus, in anoxic soils, the ferric iron reduction is controlled mainly by microbial processes (Gao et al., 2017). Although green manure incorporation

into the soil can decrease soil bulk density (Adekiya et al., 2019), WRA probably changed soil aeration in this study. Therefore, the reduction of Fe to its plant available form (Fe^{2+}) probably made it be quickly uptaken or leached by water movement as the study was *in situ*.

Effect of green manure and water retaining agent on Mn

With different application rates of WRA, the difference between the highest and the lowest value of Mn content in soils of the plots fertilized with GM varieties (*T. repens, V. villosa, M. sativa*) was statistically significant (p < 0.001) (Table 1). The trend analysis shows that the change of Mn along different WRA application rates is almost similar in plots fertilized by *T. repens* and that fertilized by *V. villosa*; Mn concentration increased as the WRA application rate increases (Figure 5). All Mn levels in plots fertilized by *T. repens* or *V. villosa* were lower than that of background properties. The present results are in line with that of Domagała-Świątkiewicz et al. (2019); the concentration of Mn in plots fertilized by *V. villosa* was observed, although it was different soil types and different study area conditions.

In the plots amended by *M. sativa*, 30 kg/ha WRA resulted in the lowest Mn concentration in soil (290 mg/kg); while the highest (426 mg/kg) was exhibited by 150 kg/ha WRA (Figure 5). Only WRA rates of 150 and 210 kg/ha resulted in a greater mean than the background properties' level of Mn. In general, the application of WRA to the field fertilized with *T. repens*, or *V. villosa*, resulted in a higher concentration of Mn in the soils than Mn concentration plots without WRA. At the
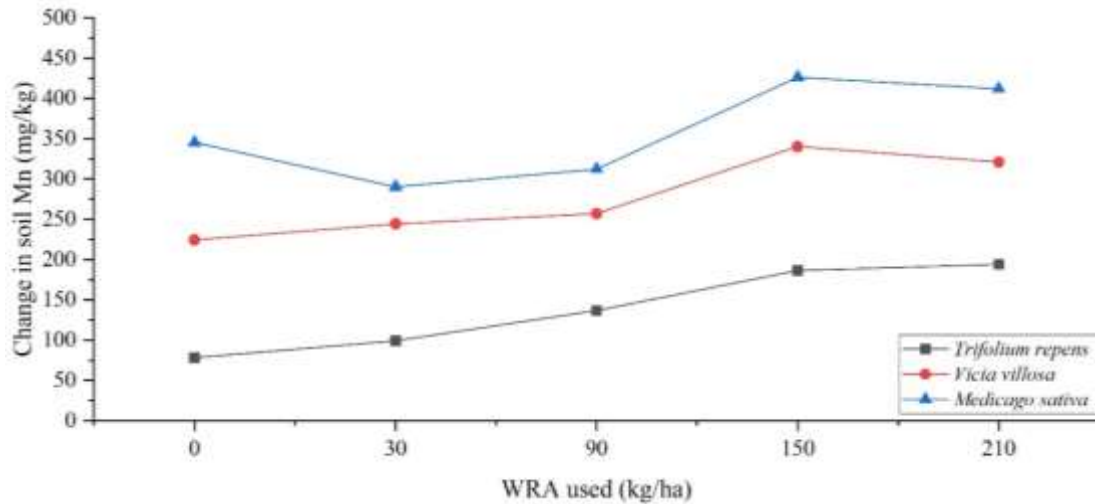
Figure 5. Levels of Mn in green manure and water retaining agent amended soil.
Source: Authors

same time, Mn showed a fluctuating change in Mn concentration in soils according to the change in WRA rate in soil fertilized by *M. sativa*.

Analogous to the present findings, Porter et al. (2004) reported that soluble soil Mn concentrations increased by 100 to 1000 fold relative to those of the control (no manure), had soil mixed with GM incubated under different conditions, the activity of redox couples was a primary factor over GM. On the other hand, Mn can form many complexes, especially in the water environment (Handa, 1970), which may be due to its ability to change the oxidation state. Mn may be present in rock and soil minerals in one or more of the three valence state; Mn^{2+}, Mn^{3+}, and Mn^{4+} (Gilkes and McKenzie, 1988). Thus, plant-induced reactions in the rhizosphere soil can significantly influence the solubility of soil Mn and the uptake of the element (Reisenauer, 1988).

Effect of green manure and water retaining agent on Cu

The difference between the lowest and the highest mean of Cu in soils fertilized by *T. repens* was statistically significant ($p = 0.002$) (Table 1). By applying WRA to soils of all plots amended by *T. repens*, soil Cu ranged between 16 and 19 mg/kg; the lowest was observed without the use of WRA and the highest was observed under 210 kg/ha WRA rate, respectively (Figure 6). By the trend analysis, the concentration of Cu increased after application of WRA in the *T. repens* fertilized plots (Figure 6). In comparison, the mean difference of Cu in soils fertilized by *V. villosa* was not significant ($p > 0.05$) (Table 1); however, the lowest was 18 mg/kg obtained

under the application of 30 kg/ha WRA and the highest was 19 mg/kg obtained under the application of 90 kg/ha WRA (Figure 6).

On the other hand, the Cu concentration from the soil amended with *M. sativa*, 90 kg/ha WRA exhibited the lowest concentration (16 mg/kg) of Cu in soils, while the highest concentration (19 mg/kg) was observed without use of WRA (Figure 6). Besides, their mean difference was statistically significant ($p = 0.001$) (Table 1). The decline of Cu levels in the soils fertilized by *M. sativa* was observed, as a result of WRA added to the studied soil (Figure 6). However, results showed a fluctuating change of Cu concentration in soils according to the change in WRA in plots fertilized by *V. villosa* or *M. sativa* (Figure 6). All Cu levels in treated soil were lower than Cu concentration in background properties. In comparison, Domagała-Świątkiewicz et al. (2019) observed lower concentration of Cu in plots fertilized by *V. villosa* compared to control plots in that study area.

Although Zhao et al. (2007) showed that the role of vegetations in the mobilization of dissolved Cu is due to their ability to enhance dissolved organic carbon concentration in the soil, the total soil Cu in our experiment has been reduced. The decline of Cu in the soil after an organic amendment was also observed (Rutkowska and Szulc, 2014), even though it was a long-term application of farmyard manure that affected Cu in the soil solution. The performance of different GM varieties on soil Cu was not similar in our experiment; *M. sativa* improved Cu than other GM legumes. The combination of WRA (90-150 kg/ha) with either *T. repens* or *V. villosa* showed better performance in soil Cu improvement than *M. sativa*. Those different effects have been observed because GM differs in chemical
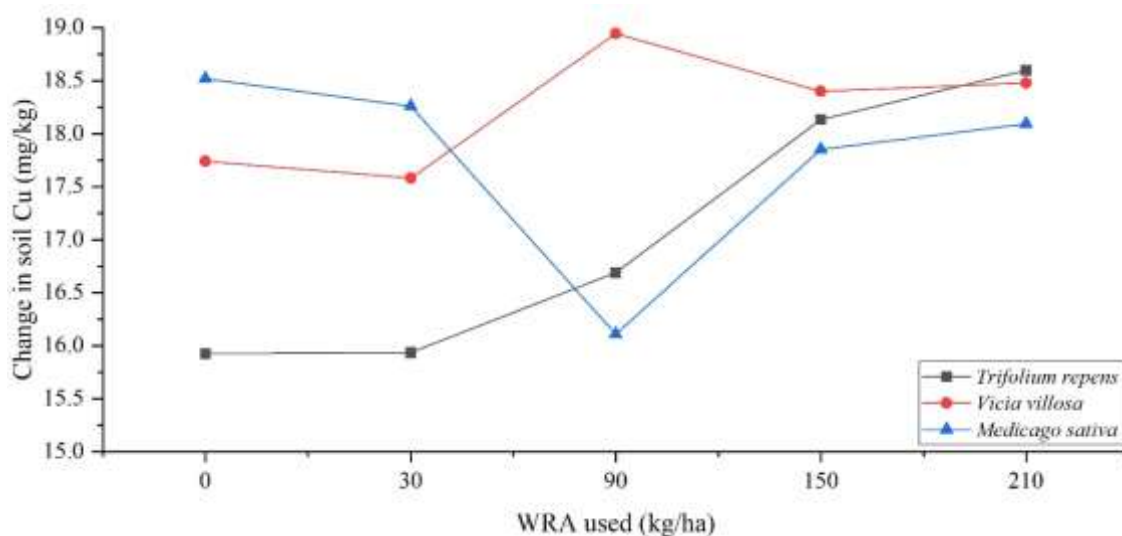
Figure 6. Levels of Cu in green manure and water retaining agent amended soil.
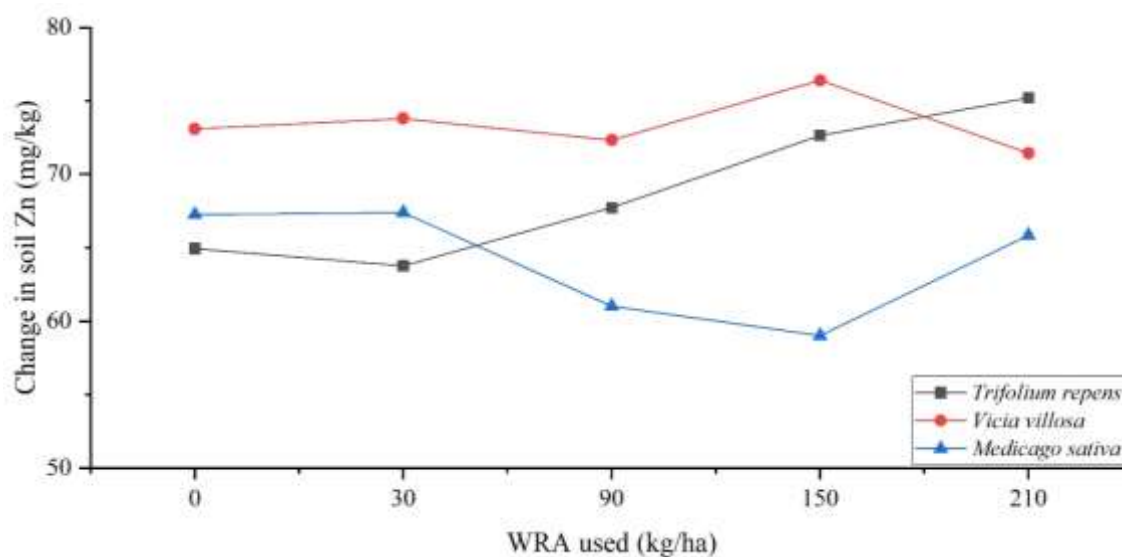Source: Authors

Figure 7. Levels of Zn in green manure and water retaining agent amended soil.
Source: Authors

composition, decomposition rate, and nutrient release to the soil (Adekiya et al., 2019).

Effect of green manure and water retaining agent on Zn

The difference between the highest and the lowest mean of Zn was statistically significant ($p < 0.001$) for soils fertilized by *T. repens* or *M. sativa*, and was not significant for soils fertilized by *V. villosa* ($p > 0.05$) (Table 1). Soil Zn concentration of the plots amended by *T. repens* ranged from 64 to 75 mg/kg observed under 30 and 210 kg/ha WRA, respectively (Figure 7); only WRA of 150 and 210 kg/ha resulted in greater Zn than the Zn concentration in background properties.

The concentration of Zn in soils from the plots amended with *V. villosa* ranged from 71 to 76 mg/kg obtained under 210 and 150 kg/ha of WRA, respectively (Figure 7); all were greater than Zn concentration in

background properties. A higher concentration of Zn in plots fertilized by *V. villosa* compared to control plots has been observed in the previous study (Domagała-Świątkiewicz et al., 2019).

On the other hand, the 150 kg/ha WRA resulted in the lowest soil Zn content (59 mg/kg) in plots amended with *M. sativa*, and 30 kg/ha WRA resulted in the highest soil Zn content (67 mg/kg) (Figure 7). Even though all mean values were lower than Zn in background properties, the plots amended by *M. sativa* and WRA greater than 30 kg/ha resulted in more reduced levels of soil Zn than the plots without WRA amendment (Figure 7). However, the changes are fluctuating according to the increase in WRA rate (Figure 7).

Green manure affects soil fertility in different ways of its application (cultivation, incorporation in soil, and mixed with soil as a dry amendment). In different purple soil (acidic, neutral, and alkaline), a high amount of root exudates of *V. villosa* significantly increased available Zn (Yang et al., 2018). Therefore, observed changes may also be due to the chemical behavior of elements by referring to the following findings. Changes in redox conditions systematically control Zn distributions and other trace elements in the soil environment (Montagne et al., 2007).

Conclusion

For the influence of GM and WRA on trace elements, a statistically significant difference was observed between concentrations of trace elements (Fe, Zn, Cu, Mo, and Mn). The concentrations of all trace elements changed after treatments application. GM increased soil Fe concentration but decreased Mo, Mn, and Cu concentration in purple soil. *T. repens* and *M. sativa* decreased soil Zn, but *V. villosa* increased the concentration of Zn in purple soil. The addition of WRA decreased soil Fe in GM fertilized plots. In plots fertilized by *T. repens*, WRA amendment increased the concentration of Mo, Mn, Cu, and Zn in soil. Although different rates of WRA affected the soil elements concentration differently, 210 kg/ha generally decreased Mo, Cu, and Zn in plots fertilized by *V. villosa*. 210 kg/ha also decreased Cu and Zn soil concentration in plots fertilized by *M. sativa*. The observed changes in trace elements concentration were not only attributed to the applied GM and WRA but also to the behavior of a specific element in soil environmental conditions. Therefore, the application of GM and WRA had a significant influence on soil trace elements in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adekiya AO, Agbede TM, Aboyeji CM, Dunsin O, Ugbe JO (2019). Green manures and NPK fertilizer effects on soil properties, growth, yield, mineral and vitamin C composition of okra (*Abelmoschus esculentus* (L.) Moench). Journal of the Saudi Society of Agricultural Sciences 18(2):218-223.

Asagi N, Ueno H (2009). Nitrogen dynamics in paddy soil applied with various 15N-labelled green manures. Plant and Soil 322(1):251-262.

Barney PA (1987). The use of Trifolium repens, Trifolium subterraneum and Medicago lupulina as overwintering leguminous green manures. Biological Agriculture and Horticulture 4(3):225-234.

Bilalis D, Karkanis A, Efthimiadou A, Konstantas A, Triantafyllidis V (2009). Effects of irrigation system and green manure on yield and nicotine content of Virginia (flue-cured) Organic tobacco (*Nicotiana tabaccum*), under Mediterranean conditions. Industrial Crops and Products 29(2-3):388-394.

Carter MR, Gregorich EG (2007). Soil sampling and methods of analysis. CRC Press.

Choi B, Daimon H (2008). Effect of hairy vetch incorporated as green manure on growth and N uptake of sorghum crop. Plant Production Science 11(2):211-216.

Crotty FV, Fychan R, Scullion J, Sanderson R, Marley CL (2015). Assessing the impact of agricultural forage crops on soil biodiversity and abundance. Soil Biology and Biochemistry 91:119-126.

Domagała-Świątkiewicz I, Siwek P, Bucki P, Rabiasz K (2019). Effect of hairy vetch (Vicia villosa Roth.) and vetch-rye (Secale cereale L.) biculture cover crops and plastic mulching in high tunnel vegetable production under organic management. Biological Agriculture and Horticulture 35(4):248-262.

Dong N, Guanglong H, Yunqi Z, Jianxun Q, Yonghao C, Yanbin H (2021). Effects of Green-Manure and Tillage Management on Soil Microbial Community Composition, Nutrients and Tree Growth in a Walnut Orchard. Scientific Reports 11(1):16882.

Eilittä M, Mureithi J, Derpsch R (2004). Green manure/cover crop systems of smallholder farmers: Experiences from tropical and subtropical regions. Dordrecht: Springer Netherlands. Available at: https://doi.org/10.1007/1-4020-2051-1

Elfanssi S, Ouazzani N, Mandi L (2018). Soil properties and agro-physiological responses of alfalfa (*Medicago sativa* L.) irrigated by treated domestic wastewater. Agricultural Water Management 202:231-240.

Fageria NK, Baligar VC, Clark RB (2002). Micronutrients in crop production. Advances in Agronomy 77:185-268.

Franco-Hermida JJ, Quintero MF, Cabrera RI, Guzman JM (2017). Determination of diagnostic standards on saturated soil extracts for cut roses grown in greenhouses. PloS One 12(5):e0178500.

Gao SJ, Cao WD, Gao JS, Huang J, Bai JS, Zeng NH, Shimizu K (2017). Effects of long-term application of different green manures on ferric iron reduction in a red paddy soil in Southern China. Journal of Integrative Agriculture 16(4):959-966.

Gilkes RJ, McKenzie RM (1988). Geochemistry and mineralogy of manganese in soils. In Manganese in soils and plants Springer, Dordrecht. pp. 23-35.

Green VS, Stott DE (2001). Polyacrylamide: A review of the use, effectiveness, and cost of a soil erosion control amendment. In The 10th International Soil Conservation Organization Meeting. Purdue University and the USDA-ARS National Soil Erosion Research Laboratory pp. 384-389.

Green VS, Stott DE, Graveel JG, Norton LD (2004). Stability analysis of soil aggregates treated with anionic polyacrylamides of different molecular formulations. Soil Science 169(8):573-581

Grüter R, Costerousse B, Bertoni A, Mayer J, Thonar C, Frossard E, Schulin R, Tandy S (2017). Green manure and long-term fertilization effects on soil zinc and cadmium availability and uptake by wheat (*Triticum aestivum L.*) at different growth stages. Science of the Total Environment 599:1330-1343.

Handa BK (1970). Chemistry of manganese in natural waters. Chemical Geology 5(3):161-165.

Hartwig NL, Ammon HU (2002). Cover crops and living mulches. Weed Science 50(6):688-699

He X, Bao Y, Nan H, Xiong D, Wang L, Liu Y, Zhao J (2009). Tillage pedogenesis of purple soils in southwestern China. Journal of Mountain Science 6(2):205-210. A

Heiberg L, Koch CB, Kjaergaard C, Jensen HS, Hansen HCB (2012). Vivianite precipitation and phosphate sorption following iron reduction in anoxic soils. Journal of Environmental Quality 41(3):938-949.

Huettermann A, Orikiriza LJ, Agaba H (2009). Application of superabsorbent polymers for improving the ecological chemistry of degraded or polluted lands. Clean–Soil, Air, Water 37(7):517-526

Huhman DV, Sumner LW (2002). Metabolic profiling of saponins in Medicago sativa and Medicago truncatula using HPLC coupled to an electrospray ion-trap mass spectrometer. Phytochemistry 59(3):347-360.

Jiang T, Lingling T, Shiqiang W, Lili D, Zaibo L, Yupeng C, Dennis CF (2010). Application of Polyacrylamide to Reduce Phosphorus Losses from a Chinese Purple Soil: A Laboratory and Field Investigation. Journal of Environmental Management 91(7):1437-1445.

Kaiser BN, Gridley KL, Ngaire BJ, Phillips T, Tyerman SD (2005). The role of molybdenum in agricultural plant production. Annals of Botany 96(5):745-754.

Karkanis A, Bilalis D, Efthimiadou A (2007). The effect of green manure and irrigation on morphological and physiological characteristics of Virginia (flue-cured) organic Tobacco (*Nicotiana tabaccum*). Available at: https://doi.org/10.3923/ijar.2007.910.919

Kim M, Song I, Kim M, Kim S, Kim Y, Choi Y, Seo M (2015). Effect of Polyacrylamide Application on Water and Nutrient Movements in Soils. Journal of Agricultural Chemistry and Environment 4(3):76-81.

Lecours N, Almeida GE, Abdallah JM, Novotny TE (2012). Environmental health impacts of tobacco farming: a review of the literature. Tobacco Control 21(2):191-196.

Lee CH, Do Park K, Jung KY, Ali MA, Lee D, Gutierrez J, Kim PJ (2010). Effect of Chinese milk vetch (*Astragalus sinicus* L.) as a green manure on rice productivity and methane emission in paddy soil. Agriculture, Ecosystems and Environment 138(3-4):343-347.

Lin C, Shihua T, Jingjing H, Yibing C (2007). Effects of plant hedgerows on soil erosion and soil fertility on sloping farmland in the purple soil area. Acta Ecologica Sinica 27(6):2191-2198.

Lisuma JB, Mbega ER, Ndakidemi PA (2021). The Effects of Cultivating Tobacco and Supplying Nitrogenous Fertilizers on Micronutrients Extractability in Loamy Sand and Sandy Soils. Plants 10(8):1597.

Ma D, Yin L, Ju W, Li X, Liu X, Deng X, Wang S (2021). Meta-Analysis of Green Manure Effects on Soil Properties and Crop Yield in Northern China. Field Crops Research 266:108146.

MacRae RJ, Mehuys GR (1985). The effect of green manuring on the physical properties of temperate-area soils. In Advances in soil science. Springer, New York, NY. pp. 71-94.

Maghchiche A, Haouam A, Immirzi B (2010). Use of polymers and biopolymers for water retaining and soil stabilization in arid and semiarid regions. Journal of Taibah University for Science 4(1):9-16.

McCauley AM (2011). Nitrogen fixation by annual legume green manures in a semi-arid cropping system. Doctoral dissertation, Montana State University-Bozeman, College of Agriculture.

Melaku S, Dams R, Moens L (2005). Determination of Trace Elements in Agricultural Soil Samples by Inductively Coupled Plasma-Mass Spectrometry: Microwave Acid Digestion versus Aqua Regia Extraction. Analytica Chimica Acta 543(1):117-23.

Miller PR, Lighthiser EJ, Jones CA, Holmes JA, Rick TL, and Wraith JM. (2011). Pea green manure management affects organic winter wheat yield and quality in semiarid Montana. Canadian Journal of Plant Science, 91(3):497-508.

Montagne D, Cornu S, Bourennane H, Baize D, Ratié C, King D (2007). Effect of agricultural practices on trace-element distribution in soil. Communications in soil science and plant analysis, 38(3-4):473-491.

Moula MS, Hossain MS, Farazi MM, Ali MH, Mamun MAA (2018). Effects of consecutive two years tobacco cultivation on soil fertility status at Bheramara Upazilla in Kushtia District. Journal of Rice Research 6:190.

Mousavi SR, Galavi M, and Rezaei M (2013). Zinc (Zn) importance for crop production—a review. International Journal of Agronomy and Plant Production 4(1): 64-68.

Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, Rascon E, Parsons JG (2001). Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa L.). Bulletin of Environmental Contamination and Toxicology 66(6):727-734.

Porter GS, Bajita-Locke JB, Hue NV, Strand D (2004). Manganese solubility and phytotoxicity affected by soil moisture, oxygen levels, and green manure additions. Communications in Soil Science and Plant Analysis 35(1-2):99-116.

Prado Wildner LD, Freitas V, McGuire M (2004). Use of green manure/cover crops and conservation tillage in Santa Catarina, Brazil. In Green Manure/Cover Crop Systems of Smallholder Farmers. Springer, Dordrecht. pp. 1-36.

Printz B, Lutts S, Hausman JF, Sergeant K (2016). Copper trafficking in plants and its implication on cell wall dynamics. Frontiers in Plant Science 7:601.

Reisenauer HM (1988). Determination of plant-available soil manganese. In 'Manganese in soils and plants'. Kluwer Academic Publishers: Dordrecht, The Netherlands. Available at: https://doi.org/10.1007/978-94-009-2817-6_7

Ren F, Zhang X, Liu J, Sun N, Wu L, Li Z, Xu M (2017). A synthetic analysis of greenhouse gas emissions from manure amended agricultural soils in China. Scientific Reports 7(1):1-13.

Roshanizarmehri M, Fotovat A, Emami H, Kehl M, Hirmas DR, Hosseinalizadeh M, Ramezanian M (2018). Combined Effects of Polyacrylamide and Nanomagnetite Amendment on Soil and Water Quality, Khorasan Razavi, Iran. Journal of Environmental Management 223:703-712.

Rutkowska B, Szulc W, Spychaj-Fabisiak E, Pior N (2017). Prediction of Molybdenum Availability to Plants in Differentiated Soil Conditions. Plant, Soil and Environment 63(11):491-497.

Salahin N, Alam MK, Islam MM, Naher L, Majid NM (2013). Effects of Green Manure Crops and Tillage Practice on Maize and Rice Yields and Soil Properties. Australian Journal of Crop Science 7(12):1901-1911.

Salim SA (2015). Effect of water-retaining agent (sky gel) on soil physical properities, growth, yield and water use efficiency of wheat (*Triticum aestivum* L.) plant. Journal of Biological Chemistry 2015;6:1

Seybold CA (1994). Polyacrylamide review: Soil conditioning and environmental fate. Communications in Soil Science and Plant Analysis 25(11-12):2171-2185.

Sumiahadi A, Acar R, Özel A (2020). Green Manure for Soil Properties Improvement. In2nd International Eurasian Conference on Science, Engineering and Technology (EurasianSciEnTech 2020).

Xiao Y, Tang J, Kuang Wang M (2016). Physicochemical properties of three typical purple soils with different parent materials and land uses in Sichuan Basin, China. Natural Resources and Engineering 1(2):59-68. Available at: https://doi.org/10.1080/23802693.2016.1258854

Xu L, Zhang D, Proshad R, Chen YL, Huang TF, Ugurlu A (2021). Effects of soil conservation practices on soil erosion and the size selectivity of eroded sediment on cultivated slopes. Journal of Mountain Science 18(5):1222-1234.

Yang LI, Xiang-Yin XI, HUANG XF, Shang-Peng YU, Sai-Nan GE (2018). Vicia villosa root exudates improve the mobilization of available P, K, Zn and Fe in three types of purple soil. In2018 International Conference on Energy Development and Environmental Protection (EDEP 2018) pp. 216-222. Atlantis Press.

Yuan XF, Jian JS, Wei XD, Ying Y (2017). Application of

Polyacrylamide Reducing Nitrate-N and Soluble P Losses from a Lou Soil in a Soil Column Experiment. International Agricultural Engineering Journal 26(1):1-8.

Zhang Y, He X, Liang H, Zhao J, Zhang Y, Xu C, Shi X (2016). Long-term tobacco plantation induces soil acidification and soil base cation loss. Environmental Science and Pollution Research 23(6):5442-5450.

Zhao LY, Schulin R, Nowack B (2007). The Effects of Plants on the Mobilization of Cu and Zn in Soil Columns. Environmental Science and Technology 41(8):2770-2775.

Zhong S, Han Z, Du J, Ci E, Ni J, Xie D, Wei C (2019). Relationships between the lithology of purple rocks and the pedogenesis of purple soils in the Sichuan Basin, China. Scientific Reports 9(1):1-13.

Zou C, Gao X, Shi R, Fan X, Zhang F (2008). Micronutrient deficiencies in crop production in China. InMicronutrient deficiencies in global crop production 2008 (pp. 127-148). Springer, Dordrecht. Available at: https://doi.org/10.1007/978-1-4020-6860-7_5