Distinguish Effect of Cu, Zn and Cd on Wheat's Growth Using Nondestructive and Rapid Minolta SPAD-502

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Abstract: This paper focused on the SPAD values response to different treatment levels of heavy metal Cu, Zn and Cd stress on growth of wheat. Random blocks design experiment has been carried out to simulate five concentration levels'heavy metal Cu, Zn and Cd respectively. The in-situ SPAD values were measured for each treatment on December 8th 2014 (seedling stage), December 13th 2014 (tillering stage), January 8th 2015(tillering stage), April 18th 2015 (elongation stage), April 30th 2015 (booting stage), May 12th 2015 (heading stage) and June 3rd 2015 (filling stage) respectively using Minolta SPAD-502. The standard deviation of SPAD in different between check plot (CK) and level i, Normalized SPAD values and their variation coefficients among five heavy metal treatments for Cd, Zn and Cu have been analyzed at different measurement time. SPAD values of CK treatment decreased slightly from seedling stage to tillering stage, followed by rapid increase to elongation stage, keeping a shoulder to heading stage, then rapid decrease to filling stage. With the increase of heavy metal treatment levels, standard deviations of SPAD values between CK and level i increased gradually, and the minimum of standard deviation appeared at booting and heading stage. The Normalized SPAD had been calculated by the SPAD ratio of heavy level i to CK. The higher Normalized SPAD, the lower Normalized SPAD value with the minimum at booting and heading stage. Variance coefficients of Normalized SPAD of Cd treatments at seedling stage and tillering stages lower than Zn and Cu treatments. Normalized SPAD among Cd, Cu and Zn treatments had lowest and similar variance at booting stage and heading stages.

1. Introduction

Heavy metals (HM) means that some elements which have specific weights of more than 5 g/cm^3 (M. M. Lasat, 2000). Industrial revolution causes high anthropogenic emission of heavy metals into soil and water (Ayres, 1992).Plants' rootsare the primary contact site for the metal ions (Eisenreich W,1996). As for plant metabolism, HMs such as Cu and Zn are essential microelement. But when present in excess, Cu and Zn also can become extremely toxic just like low levels of Cd, Hg, and Pb, (Dharmendra K. Gupta,2013). Incorporated various techniques of analysis have been used to quantify the effects of HMs on plant including leaf injury with visual assessment, biochemical assessment and spectral measurements.

The SPAD-502 meter uses two light-emitting diodes (650nm and 940nm) and a photodiode detector. The light-emitting diodes and photodiode detector measure transmission sequentiallythrough leaves (John Markwell, 1987). SPAD-502 has been utilized for determining the nitrogen status, chlorophyll content, thickness of leaf , succulence of leaf , specific leaf area, leaf mass, leaf water content, photosynthesis, and so on.(Campbell RJ ,1990; Castelli F, 1996；Azia F, Stewart KA 2001; Balasubramanian, V.,2000; Richardson AD,2002; Francesco Giunta,2002; Wang QB ,2004; Jifon JL,2005; Alena Torres Netto, 2005, Uddling J.,2007; Marenco R.A., 2009). SPAD readings may represent a useful screening criterion of crop growth status. And SPAD-502's utilization saves much more time and resources than traditional destructive methods (Alena Torres Netto,2005). SPAD readings come from the leaves' transmitted spectra at 650nm and 940nm. It has been recognized as a simple and portable diagnostic tool to measure the greenness or chlorophyll content of leaves, thus it may become useful tool being used to assess the stress effects on plant growth.

The plant leaves had very strong enrichment ability for some elements and plant trace elements had a strong influence growth status of leaves, which can response to transmitted spectra of leaves at red and infrared bands used by SPAD. Few publications reported on SPAD as a tool to detect heavy metal stress of wheat. This research was

undertaken to quantify the responses of SPAD readings of wheat among different heavy metal treatments. The objective of this present study was to find the potential of SPAD -502 assessing heavy metal Cu, Zn and Cd stress on wheat.

2. Materials and methods

The field plots (genotype of wheat is called Jimai 22) was treated with heavy metal Cu ($CuSO_4$ $5H_2O$), Zn （$ZnSO_4$ $7H_2O$）and Cd （$3CdSO_4.8H_2O$） at five-level treatments (see table1) and three repeated trials. Seed on 17th October 2014. Plots with area of $2\times3m^2$had been arranged in a randomized block consisting of same fertilizer applications (figure1). *In-situ* nondestructive SPAD values were measured by Minolta SPAD-502 on December 8th 2014 (seedling stage), December 13th 2014 (seedling stage), January 8th 2015(tillering stage), April 18th 2015 (elongation stage), April 30th 2015 (booting stage), May 12th 2015 (heading stage) and June 3rd 2015 (filling stage) respectively. Middle of the upper full expanded leaves had been chosen to measure the SPAD values. *In-situ* Minolta SPAD-502 was taken 20-30 values per block.

Table1. The concentration treatment levels of Cd^{2+} Zn^{2+} Cu^{2+} (mg/kg)

	Ck	L1	L2	L3	L4
Cd^{2+}	0.00	1.00	3.00	5.00	8.00
Zn^{2+}	0.00	250.00	500.00	750.00	1000.00
Cu^{2+}	0.00	100.00	300.00	600.00	900.00

Figure1. Distribution diagram of field plots (PVC sheets and earthen rows had been put among different blocks in order to block heavy metals diffuse among adjacent blocks)
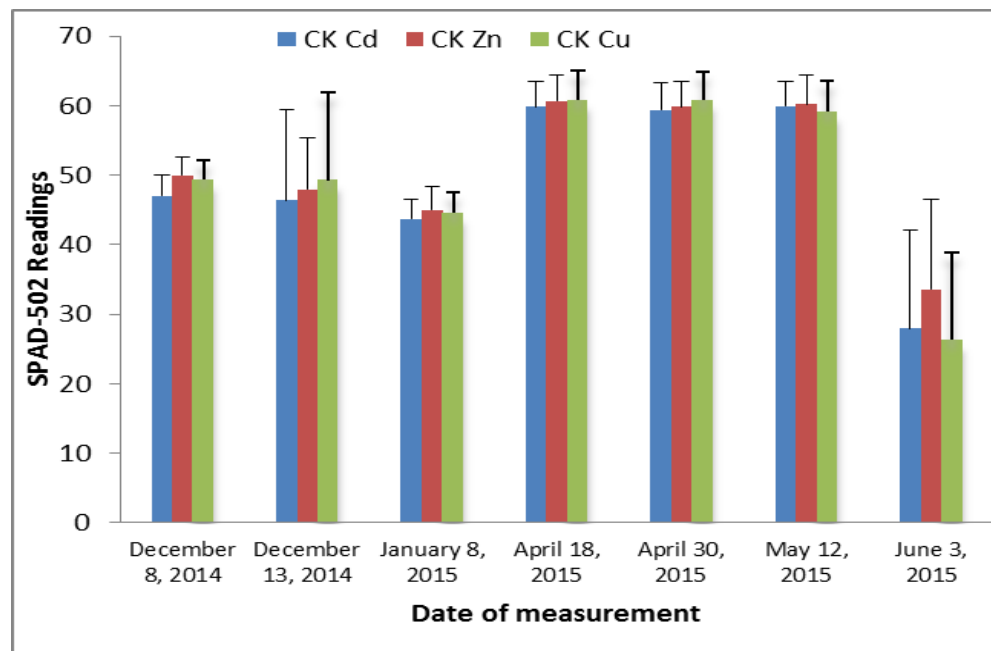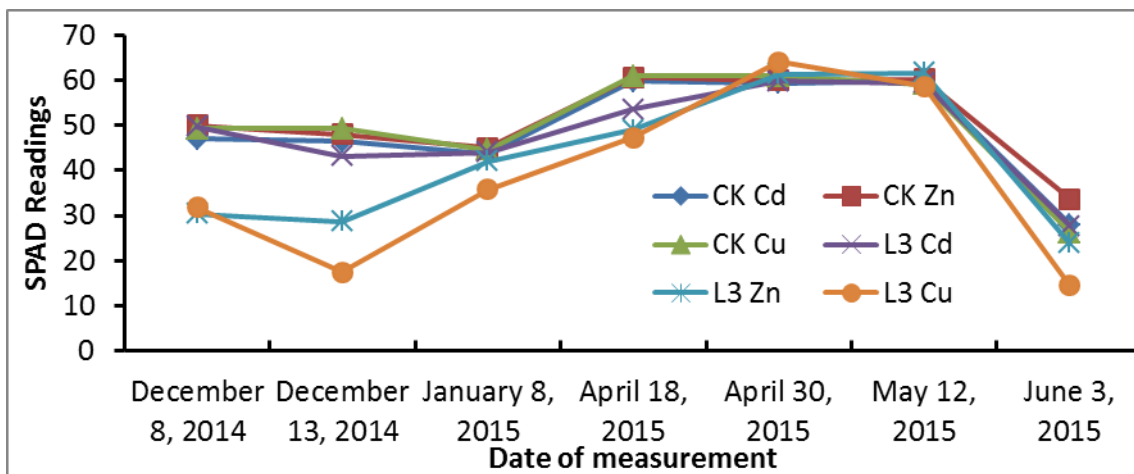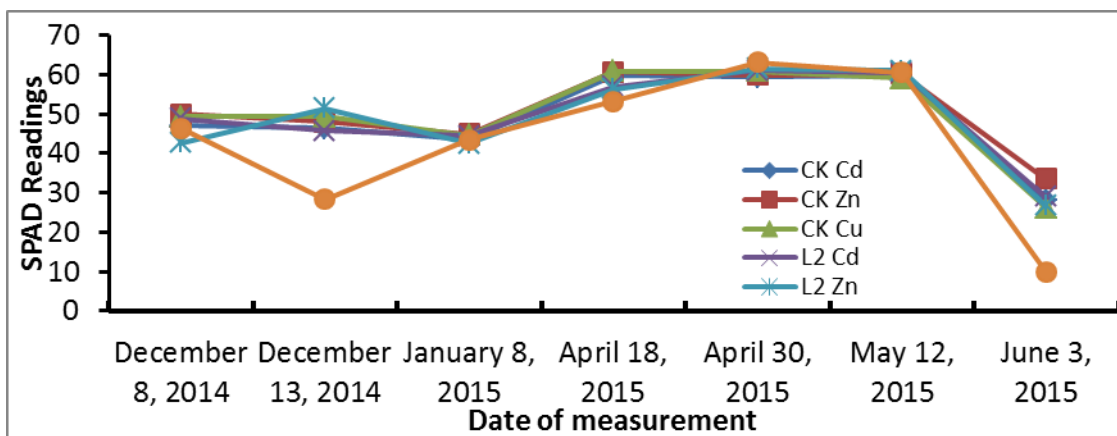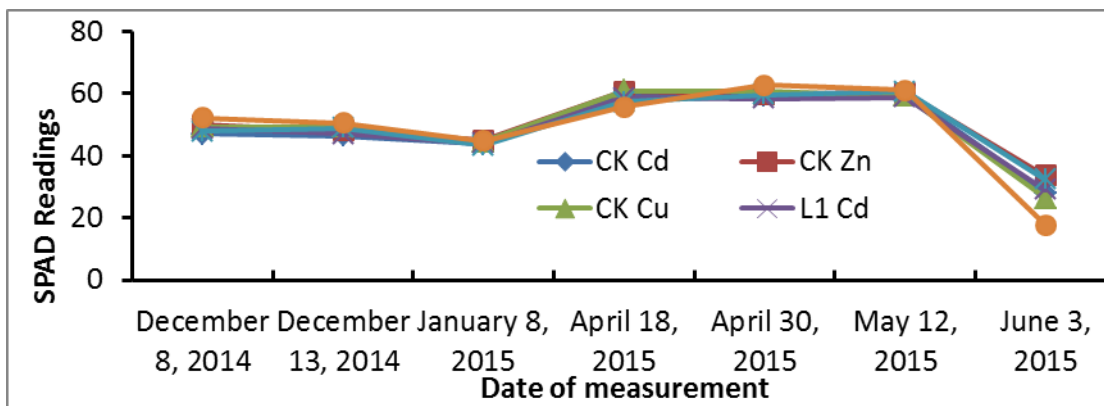
3. Results and discussion

Figure2. SPAD readings among different measurements

SPAD values of check plot (CK) for Cd, Zn and Cu treatments changed as follow: slight decrease from seeding stage on Dec. 8th, 2014 to tillering stage on Jan 13th, 2015. Then the SPAD values increase rapidly from tillering stage on Jan 13th, 2015 to elongation stage on April 18th, 2015. There is a shoulder from elongation stage on April 18th, 2015 to heading stage on May 12th, 2015, then decrease rapidly again, which indicated that different SPAD values appeared different growth stages of wheat under normal growing status. The highest SPAD values appeared at elongation and heading stages (Figure2).
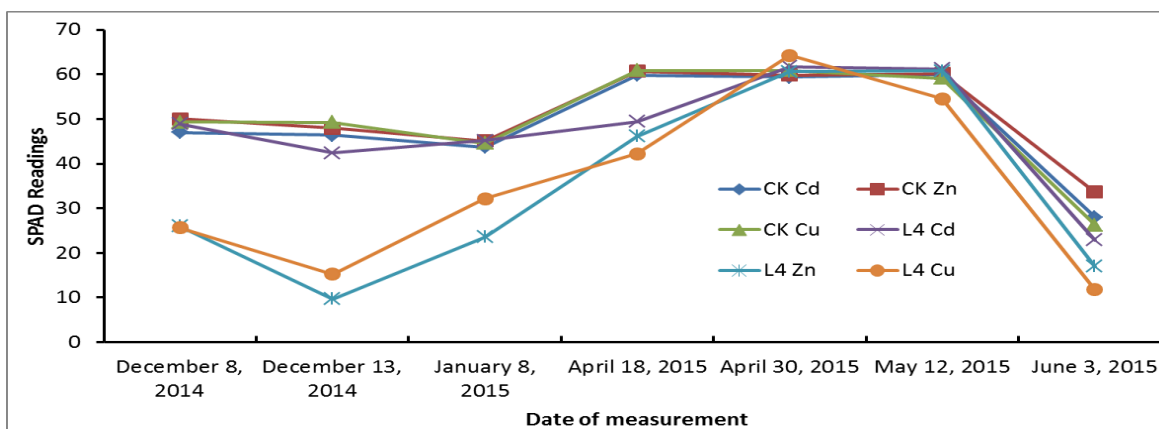
Figure 3. The dispersion degree increase gradually according to the sequence between CK and L1, CK and L2, CK and L3, CK and L4

Compare the different between SPAD readings measured from check plot (CK) and heavy metal treatment level 1 (L1), level2 (L2), level 3 (L3) and level 4 (L4) respectively at the whole growth stages. As the heavy metal treatment levels increased from L1 through L4, the dispersion degree increase gradually according to the sequence between CK and L1, CK and L2, CK and L3, CK and L4 (figure3 a,b,c,d). The dispersion degree also could be indicated by standard deviations of SPAD readings between CK and Level i (i =1,2,3,4 respectively). With the increase of heavy metal treatment levels, standard deviations of SPAD values increased step by step followed by the sequence between CK and L1 < CK and L2 < CK and L3 < CK and L4(figure 4), which also can be proved by the SPAD value ratio of different level to CK (figure5).
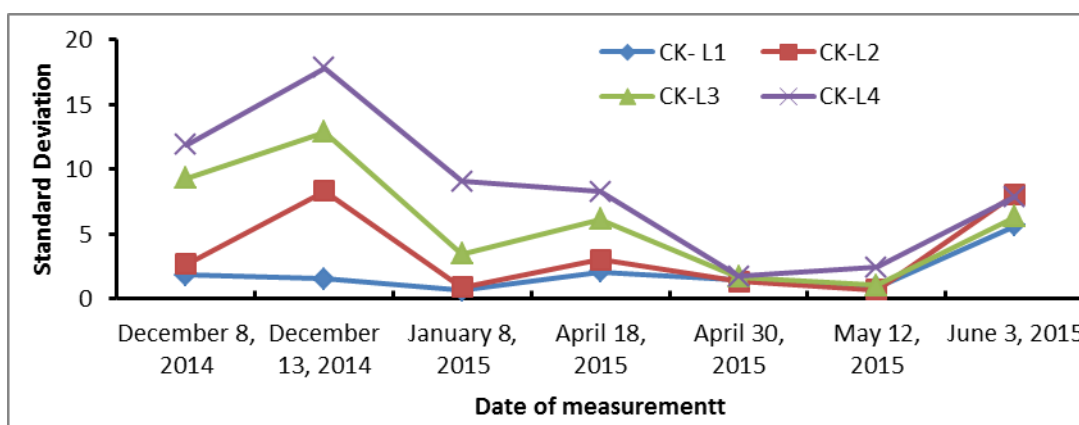

Figure 4. Compare standard deviation of SPAD values between CK and Level i (i =1,2,3,4 respectively)

CK-Li means standard deviation between CK and heavy metal treatment level i.

SPAD reading had been normalized using the ratio of different level to CK, which called Normalized SPAD. The higher heavy metal level, the lower ratio value appeared at whole growth stages. Minimum difference of both the ratio and the standard deviation appeared on April 30th 2015 (booting stage) and May 12th 2015 (heading stage) (figure5), which indicated that heavy metals have the least influence on booting stage and heading stage of wheat reflected by the changes of SPAD readings (figure5). The phenomenon also can be proved by variance coefficients of Normalized SPAD. Variance coefficients of Normalized SPAD of Cd treatments on December 8th 2014 (seedling stage), December 13th 2014 (tillering stage) and January 8th 2015(tillering stage) less than Zn and Cu treatments. Normalized SPAD among Cd, Cu and Zn treatments had lowest and similar variance on April 30th 2015 (booting stage) and May 12th 2015 (heading stage) (figure 6).
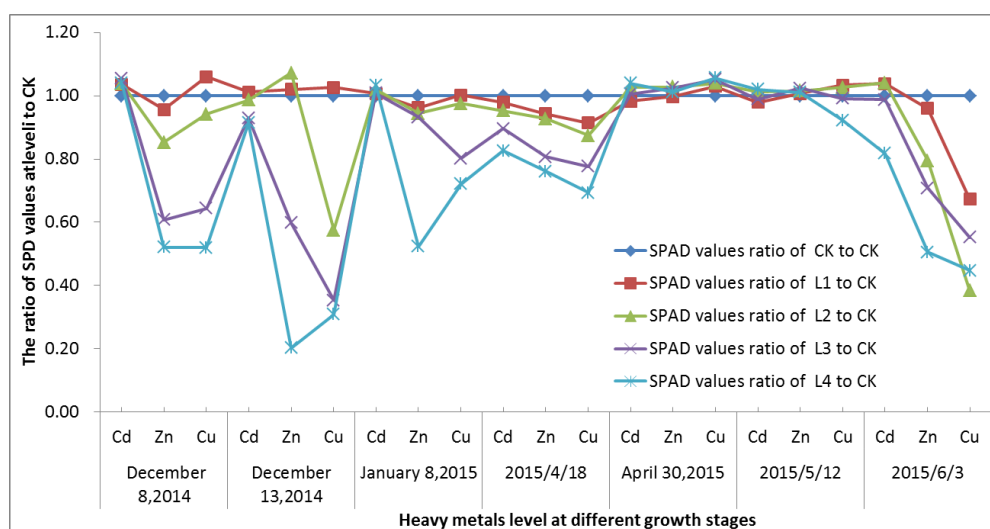

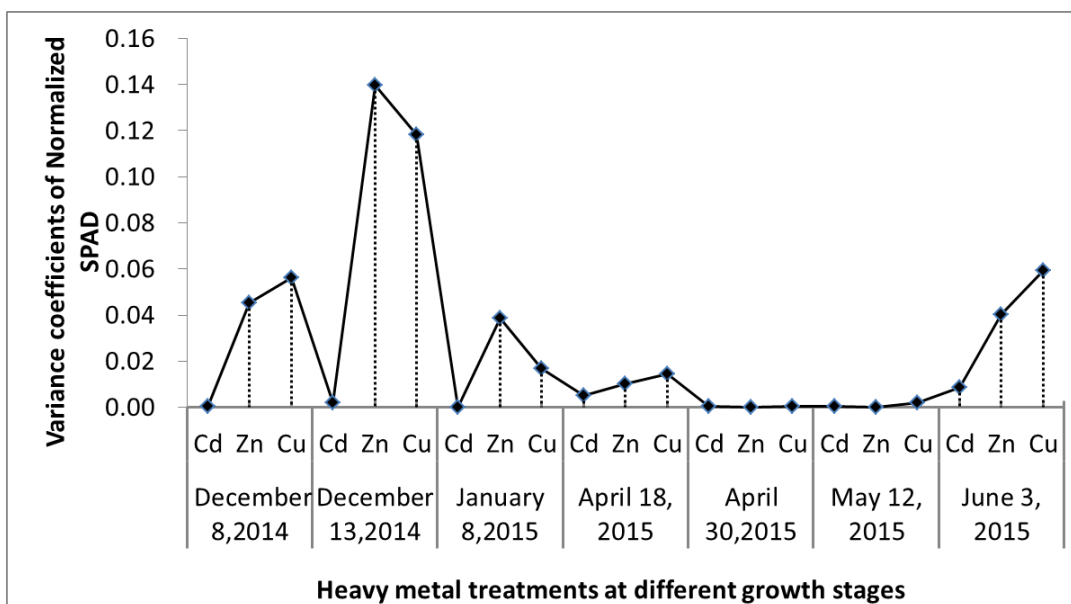Figure 5. SPAD value ratio of different level to CK

Figure 6. Variance coefficients of Normalized SPAD of heavy metal treatments at different growth stages

4. Conclusion

Minolta SPAD-502 can be utilized to distinguish the effects of Cd, Cu, Zn on wheat as a nondestructive and rapid tool. The key findings from this research as follow:

(1) Different SPAD values appeared different growth stages of wheat under normal growing status. The highest SPAD values appeared at elongation and heading stages, which indicated the most vigorous growth stages of wheat are elongation and heading stages.

(2)The SPAD readings different and their standard deviations between CK and heavy metal treatment level i (CK and L1, CK and L2, CK and L3, CK and L4) showed that with the increased heavy metal treatment levels, the dispersion degree and standard deviations increased gradually, which indicated that the higher heavy metal concentration treatment, the more influence over wheat's growth.

(3) Higher heavy metal level had lower Normalized SPAD values. Minimum difference of Normalized SPAD and standard deviation between CK and level i appeared at booting stage and heading stage. And Normalized SPAD among Cd, Cu and Zn treatments had lowest and similar variance at booting and heading stage. These results indicated that heavy metals have the least influence over wheat's growth at booting stage and heading stage.

(4) Variance coefficients of Normalized SPAD of Cd treatments at seedling stage and tillering stage lower than Zn and Cu treatments, which indicated the influence of Cd over wheat's growth is less than heavy metal CU and Zn.

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