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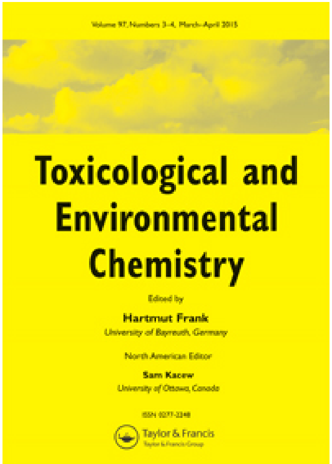

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A pilot study on the effect of Cu, Zn, and Cd on the spectral curves and chlorophyll of wheat canopy at tiller stage

Jinheng Zhang[a], Lusheng Zeng[b], Yonghong Sun[c], Chaoyu Song[c], Hui Wang[ab], Jianmei Chen[c] & Chandrashekhar Biradar[d]

[a] Institute of Eco-Environment and Agriculture Information, College of Environment and Safety Engineering, Qingdao University of Science and Technology, Qingdao, China

[b] College of Resource and Environment, Qingdao Agricultural University, Qingdao, China

[c] Qingdao Agricultural Science Research Institute, Qingdao, China

[d] International Center for Agricultural Research in Dry Areas (ICARDA), Amman, Jordan

Published online: 15 Jul 2015.

To cite this article: Jinheng Zhang, Lusheng Zeng, Yonghong Sun, Chaoyu Song, Hui Wang, Jianmei Chen & Chandrashekhar Biradar (2015) A pilot study on the effect of Cu, Zn, and Cd on the spectral curves and chlorophyll of wheat canopy at tiller stage, Toxicological & Environmental Chemistry, 97:3-4, 454-463, DOI: 10.1080/02772248.2015.1050199

To link to this article: http://dx.doi.org/10.1080/02772248.2015.1050199

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Toxicological & Environmental Chemistry, 2015
Vol. 97, Nos. 3–4, 454–463, http://dx.doi.org/10.1080/02772248.2015.1050199

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Jinheng Zhang[a]*, Lusheng Zeng[b], Yonghong Sun[c], Chaoyu Song[c], Hui Wang[a,b], Jianmei Chen[c] and Chandrashekhar Biradar[d]

[a]*Institute of Eco-Environment and Agriculture Information, College of Environment and Safety Engineering, Qingdao University of Science and Technology, Qingdao, China;* [b]*College of Resource and Environment, Qingdao Agricultural University, Qingdao, China;* [c]*Qingdao Agricultural Science Research Institute, Qingdao, China;* [d]*International Center for Agricultural Research in Dry Areas (ICARDA), Amman, Jordan*

(*Received 28 March 2015; accepted 29 April 2015*)

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The aim of this study was to investigate canopy spectral reflectance responses to different levels of heavy metals copper (Cu), zinc (Zn), and cadmium (Cd) induced stress. Random blocks design experiment was conducted to simulate Cu, Zn, and Cd at five concentration levels. Chlorophyll and visible and near infrared canopy reflectance were measured for each treatment 56 days after seeding using chlorophyll meter SPAD-502 and spectroradiometer, respectively. New vegetative indices termed ratio of inclination angles (RIA), MERIS (the medium resolution imaging spectrometer) terrestrial chlorophyll index (MTCI), and normalized difference vegetation index (NDVI) were used to assess the response of canopy spectral reflectance to different heavy metal levels. Significant spectral variability especially in blue, red, and near infrared reflectance was observed for different heavy metal treatments. One-sample test showed significant difference for NDVI, MTCI, and RIA among five-level treatments at the 0.01 level. Correlation analysis demonstrated that the two vegetative indices (RIA and MTCI) were significantly correlated with chlorophyll meter values for Cd, Cu, and Zn treatments. However, NDVI was only significantly correlated with chlorophyll meter values for Cu and Zn treatments. Linear regression analysis also illustrated that RIA and MTCI were potential indices for predicting chlorophyll concentration with significant *F* test under Cu-, Zn-, and Cd-mediated stress.

Keywords: Cu; Zn; Cd; canopy spectral reflectance; chlorophyll meter; vegetative index

Introduction

Heavy metals (HMs) are conventional elements with properties like ductility, conductivity, stability as cations, ligand specificity, and an atomic number >20. HMs such as copper (Cu) and zinc (Zn) are essential micro-nutrients for plant metabolism but when present in excess, these elements produce adverse effects., Low levels of nonessential HMs such as cadmium (Cd), mercury (Hg), and lead (Pb) are known to exert toxic manifestations (Dharmendra, Francisco, and Palma 2013). Attempts to quantify the effects of HMs on plants incorporated various techniques of analysis including visual assessment of leaf injury, biochemical assays, and spectral measurements. Remote sensing techniques, which are based on the measurement of the reflected spectra by crop canopies at different

*Corresponding author. Email: zhangjinheng1973@gmail.com

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wavelengths, reduce laborious field sampling, sample processing procedures and improve efficiency; thus these methodologies may become useful tools in the assessment of stress effects on crop growth (Zhang, Han, and Li 2010). Such spectral based techniques have been applied extensively in the field of environmental and ecosystems monitoring. Several relationships between biospectral response of crops and growth factors were elucidated based upon reflectance obtained from handheld and aircraft-mounted sensors providing images in visible and other parts of the electromagnetic spectrum (Broge and Mortensen 2002; Carlos, Lianne, and Pierre 2001; Takihashi et al. 2000; Card et al. 1988; McLellan, Aber, and Martin 1991; Kokaly and Clark 1999; Curran et al. 1992; Zhang et al. 2006; Goel et al. 2003).

Plant leaves possess potent enrichment ability toward some elements such as Mg, Ca, K, Na, Fe, AI, Mn, V, Zn, and Cr. Further, plant trace elements exert a strong influence on the "blue-shift" strength and reflectance of visible bands (Wang 2000). *In situ* as well as space-borne spectral data such as data Landsat TM and ETM indicated that the normalized difference vegetative index (NDVI) may be used to distinguish metal stressed plants. The NDVI showed significant negative correlation with increase in soil Cu concentrations followed by other elements (He and Zhang 2014). Elevation in plant stress with increase in biosolid dose was evident in radish plants through significant reduction in NDVI (Maruthi et al. 2014). Another vegetation index MERIS (medium resolution imaging spectrometer) terrestrial chlorophyll index (MTCI) utilization data in three red/near infrared wavebands centered at 681.25, 708.75, and 753.75 nm (bands 8, 9, and 10 in the MERIS standard band setting) has been served as a standard level-2 product of the European Space Agency (Dash and Curran 2007). However MTCI has not yet been applied to detection of plant HM-mediated stress at field level. Another new vegetative index ratio of inclination angles (RIA) was constructed based upon three-inflection point reflectance at 680 nm (R680), 700 nm (R700), and 750 nm (R750) to compare with NDVI and MCTI.

This investigation was undertaken to quantify the relationship between three indices (RIA, MCTI, and NDVI) in detecting HM exposure and chlorophyll concentration in wheat. The objective of this present study was to determine which vegetative index was more sensitive for assessing nutrient stress condition associated with key elements such as Cu, Zn, and Cd on wheat crops.

Materials and methods

The field experiment genotype (Jimai 22) was treated with HMs Cu, Zn, or Cd at five concentrations (see Table 1) and three applications during wheat tillage stages (October 2014). Plots were arranged in a randomized block and consisted of same fertilizer applications level with an area of $2 \times 3\ m^2$. *In situ* canopy spectral reflectance between 350 and 1100 nm was measured using a spectroradiometer Avantes (350–1100 nm) under

Table 1. The concentration treatment levels of Cd^{2+}, Zn^{2+}, and Cu^{2+} (mg/kg).

	Ck	L1	L2	L3	L4
Cd^{2+}	0	1	3	5	8
Zn^{2+}	0	250	500	750	1000
Cu^{2+}	0	100	300	600	900

clear weather conditions and as close to solar noon as possible at 56 days after seeding. The sensor, a fiber-optic of 25° field of view, was held directly from 70 cm above the canopy. Nondestructive chlorophyll meter SPAD-502 measurement was used to determine indicative chlorophyll concentration. *In situ* chlorophyll meter SPAD-502 examined 20−30 values per block in order to indicate chlorophyll concentration. Experiments were taken on 13 December 2014 (56 days after seeding, tiller stage).

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Results

The canopy spectral reflectance of wheat following Cu, Zn, or Cd treatments was measured after 56 days. It was decided to investigate the spectral effects of HM-induced stress using wavelengths between 350 and 1000 nm. The canopy spectral reflectance of different Cu, Zn, and Cd treatments showed distinct changes in the curve shape, especially at absorption of blue and red and reflectance of near infrared. The canopy spectral reflectance curves were altered in the order of level CK through L4 for Cu and Zn treatments (Figure 1(a) and 1(b)). There was no significant change in canopy spectral reflectance curves for Cd treatment (Figure 1(c)).

Differences in HM concentration influenced spectral reflectance. In the visible region, red absorption area displayed differences among different HM concentrations, which may be attributed predominantly to variation in chlorophyll absorption. Differences in reflectance between treatments depended on differences in chlorophyll concentration. The characteristic reflectance at wavelengths about 500 and 680 nm in the curves of Figure 1(a) and 1(b) was almost absent at higher Zn^{2+} and Cu^{2+} concentrations. The notable canopy spectral reflectance difference was observed at near infrared wavelengths among HM treatment levels. Alterations in reflectance blue and red wavelengths corresponded to two chlorophyll absorption bands and were characteristics of healthy green vegetation. Canopy pigment damage reduces the ability of the pigments to absorb red and blue light, which often are associated with the pollutant fraction responsible for producing foliar injury. Spectral reflectance vegetative indices based on simple calculation among the given wavelength reflectance were used in the assessment of plant characteristics. Three-inflection point reflectance at 680 nm (R680), 700 nm (R700), and 750 nm (R750) as illustrated in Figure 1 was used to calculate MTCI according to formula (1) and RIA formula (2) and Figure 2. In addition, near infrared at 800 nm and red at 680 nm were used to calculate NDVI formula (3).

$$\mathrm{MCTI} = \frac{\mathrm{R750} - \mathrm{R700}}{\mathrm{R700} - \mathrm{R680}} \tag{1}$$

$$\text{Ratio of inclination angles(RIA)} = \frac{\mathrm{Tan}\beta 1}{\mathrm{Tan}\beta 2} = \frac{(\mathrm{R750} - \mathrm{R700})/(750 - 700)}{(\mathrm{R700} - \mathrm{R680})/(700 - 680)} \tag{2}$$

$$\mathrm{NDVI} = \frac{\mathrm{R800} - \mathrm{R680}}{\mathrm{R800} + \mathrm{R680}} \tag{3}$$

One-sample test showed significant difference at the 0.01 level with RIA, MCTI, and NDVI in the presence of five-level treatments (Table 2).

The influence of Cu, Zn, and Cd on canopy reflectance spectral vegetative indices was investigated (Table 3). Both RIV and MTCI were negatively correlated with Cu^{2+}, Zn^{2+}, and Cd^{2+} concentration at 0.01 or 0.05 levels except for NDVI for Cd and Cd^{2+}. Thus it

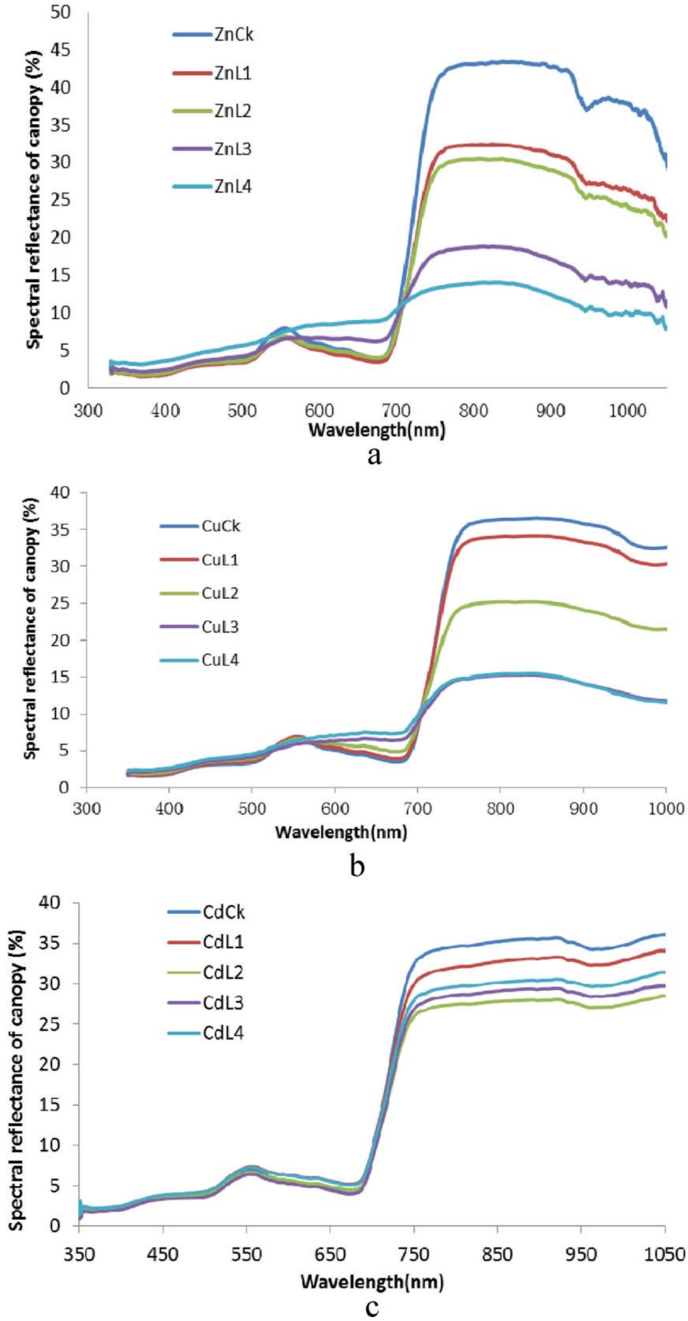

Figure 1. Canopy spectral reflectance curves for wheat crop of concentration treatments of Zn, Cu, and Cd (CK and L1−L4 indicate treatment levels and control, respectively, see Table 1).

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appeared that RIV and MTCI demonstrated potential to assess wheat injury by Cu, Zn, and Cd. NDVI might be used to determine wheat injury by Cu and Zn but not Cd.

RIA and MCTI were significantly correlated with chlorophyll concentration with SPAD-502 values at 0.01 level for Cd, Cu, and Zn treatments (Table 4). NDVI was

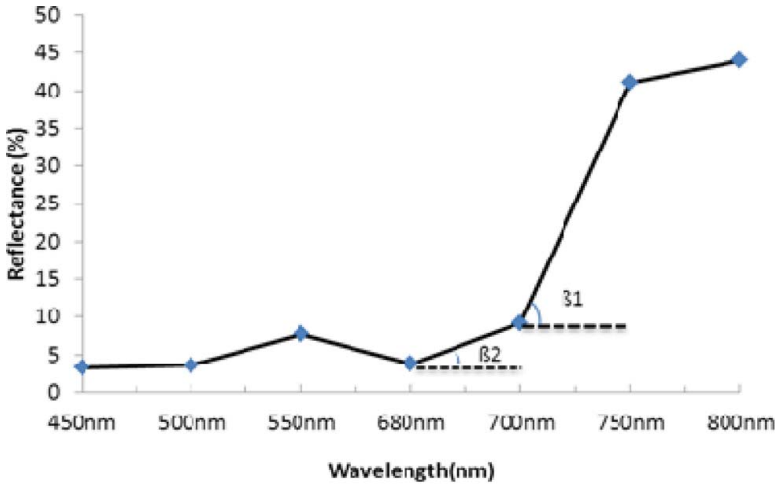

Figure 2. Two angles in the vegetative index of RIA (ratio of inclination angles).

significantly correlated with chlorophyll meter SPAD -502 values at 0.01 level for Zn and 0.05 level for Cu. However NDVI was insignificantly associated with SPAD-502 values for Cd. Thus it appeared that RIA and MCTI might be utilized to detect chlorophyll concentration induced by HM exposure. Linear regression models predicting chlorophyll meter values using RIA and MTCI were significant by *F* test at 0.01 level for Zn and Cu and 0.05 level for Cd exposure, respectively (Figure 3).

Discussion

The canopy spectral reflectance of different Cu, Zn, and Cd treatments showed distinct alterations especially at blue, red, and reflectance of near infrared wavelength. The canopy spectral reflectance curves changed significantly in the order of level CK (0 mg/kg) through to L4 for Cu and Zn exposure but there was no significant change in order of canopy spectral reflectance curves for Cd. The basis for these findings may be attributed to change in plant cellular structure under different HM stress conditions. Differences in

Table 2. Significant differences of canopy reflectance vegetative index for five-level treatments.

Vegetative index	Heavy metal	*N*	Mean	Std. deviation	Std. error mean	*t*	df	Sig.
RIA	Zn	5	1.7754	0.7453	0.3333	5.3270	4	0.0060**
	Cu	5	1.4772	0.6279	0.2808	5.2600	4	0.0060**
	Cd	5	1.8342	0.0769	0.0344	53.3280	4	0.0000**
MTCI	Zn	5	4.4386	1.8633	0.8333	5.3270	4	0.0060**
	Cu	5	3.6930	1.5698	0.7020	5.2600	4	0.0060**
	Cd	5	4.5854	0.1923	0.0860	53.3210	4	0.0000**
	Zn	5	0.6234	0.2644	0.1182	5.272	4	0.0060**
NDVI	Cu	5	0.6048	0.2225	0.0995	6.077	4	0.0040**
	Cd	5	0.7341	0.0342	0.0153	47.976	4	0.0000**

**The mean difference is significant at 0.01 level.

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Table 3. Correlation analysis between vegetative indices (RIA, MTCI, and NDVI) and heavy metal treatment.

Pearson correlation	RIA of heavy metal treatment			MTCI of heavy metal treatment			NDVI of heavy metal treatment		
	ZN	Cu	Cd	ZN	Cu	Cd	ZN	Cu	Cd
Correlation coefficient	−0.927*	−0.976**	−0.930*	−0.927*	−0.976**	−0.930*	−0.924*	−0.979**	−0.568
Sig. (2-tailed)	0.023	0.005	0.022	0.023	0.005	0.022	0.025	0.004	0.318
N	5	5	5	5	5	5	5	5	5

Note: * and ** indicate significance at the 0.05 and 0.01 levels, respectively.

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HM concentration influenced spectral reflectance with a characteristic alteration in reflectance blue and red wavelengths corresponding to two chlorophyll absorption bands, indicative of healthy green vegetative state. Canopy pigment damage reduces the ability of pigments to absorb red and blue light, which may account for HM concentrations being responsible for causing foliar and chloroplast injury. Andres and Andrea (2002) reported that when not sufficiently detoxified rapidly Cd may trigger, via disturbance of the cellular redox control, a sequence of reactions leading to growth inhibition, stimulation of secondary metabolism, lignification, and finally cell death. Xiao et al. (2008) noted that chloroplast increased in wheat leaves under low concentration Cu^{2+}-mediated stress. Further, under high Cu concentration exposure swollen chloroplast and disrupted chloroplast membrane and cascade structure damaged of chloroplast stroma occurred. Experiments focusing on cell ultrastructure are currently underway to determine the different spectral curves in our project. Based on the characteristic changes in reflectance, especially three-inflection point reflectance at 680, 700, and 750 nm, canopy spectral reflectances of 680, 700, and 800 nm were used to construct the vegetative indices of NDVI, RIV, and MTCI. The MTCI was used to determine chlorophyll levels (Dash et al. 2010), salt stress in coastal vegetation (Curran, Dash, and Llewellyn 2007), phenology of vegetation (Doreen et al. 2011), and herbicide concentration (Dash and Curran 2006). MTCI and NDVI were also utilized to detect the effects of other pollutants on plant, such as estimating the injury of rice by air sulfur dioxide (SO_2) pollutant (Zhang, Han, and Li 2010). Although MCTI was significantly correlated with chlorophyll concentrations, there was no marked association with SO_2 levels (Zhang, Han, and Li 2010). There were no apparent reports

Table 4. Correlation analysis between vegetative indices (RIA and MTCI) and SPAD values.

	RIA of heavy metal treatment			MCTI of heavy metal treatment			NDVI of heavy metal treatment		
	Zn	Cu	Cd	Zn	Cu	Cd	ZN	Cu	Cd
Correlation coefficient	0.975**	0.98**	0.934**	0.975**	0.98**	0.934**	0.986**	0.954*	0.213
Sig. (2-tailed)	0.005	0.003	0.002	0.005	0.003	0.002	0.002	0.012	0.637
N	5	5	5	5	5	5	5	5	5

Note: * and ** indicate significance at the 0.05 and 0.01 levels, respectively.

regarding MTCI on HM-induced stress on plants. In this investigation both RIV and MTCI were significantly negatively correlated with Cu^{2+}, Zn^{2+}, and Cd^{2+} concentration, and significantly positively correlated with chlorophyll levels using SPAD values. NDVI

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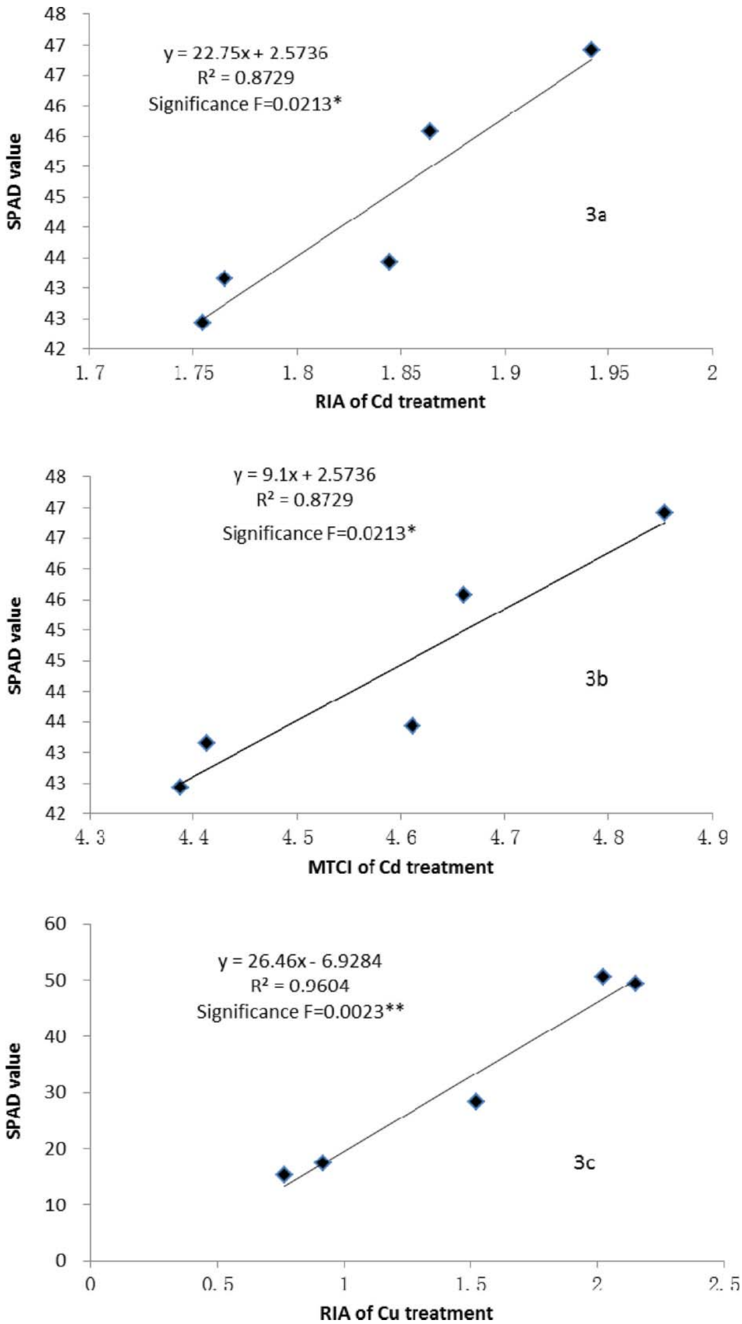

Figure 3. Quadratic polynomial regression models predicting chlorophyll concentration using RIA and MTCI.

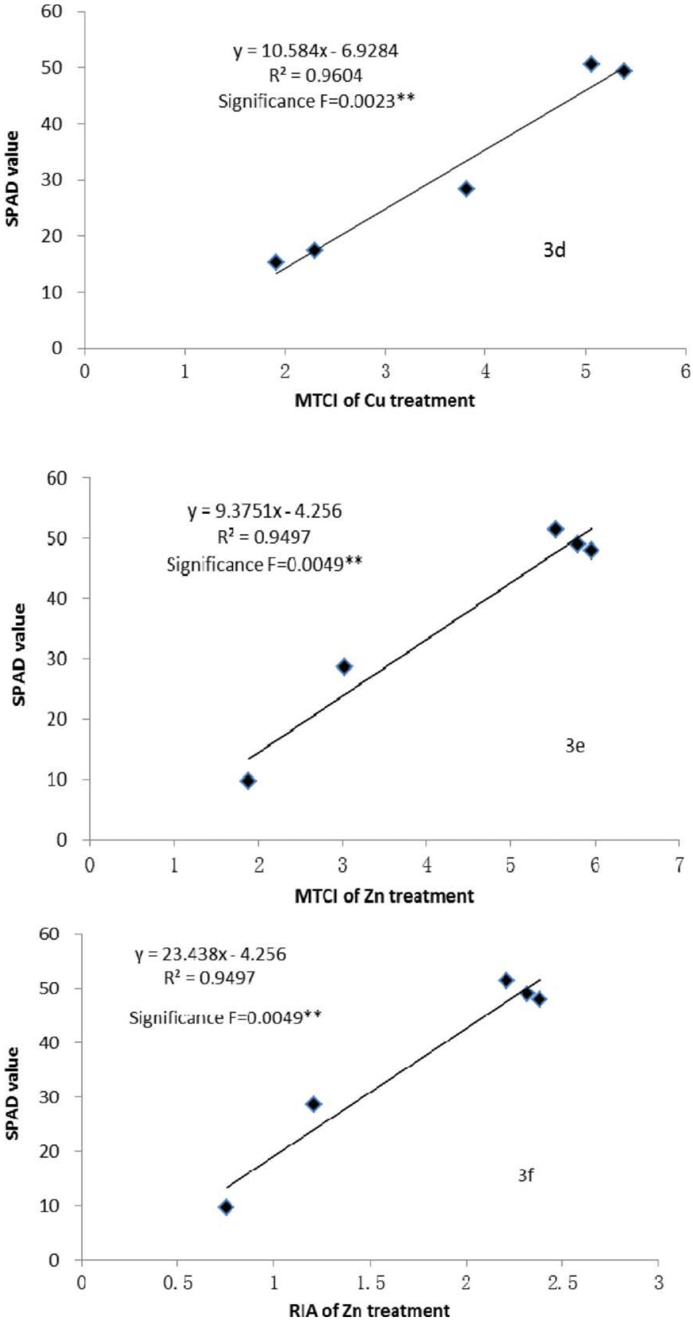

Figure 3. (*Continued*)

only was significantly negatively associated with Cu^{2+}, Zn^{2+} but not with Cd^{2+}. Data showed that NDVI was significantly correlated with Cu^{2+} or Zn^{2+} exposure and SPAD-502 values for Cu and Zn treatment. A significant correlation was not detected for Cd treatment. Thus it appeared that MTCI and the new index of RIV possess similar potential to estimate wheat injury by Cu, Zn, and Cd.

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Conclusion

The key findings drawn from this investigation are as follows: three vegetative indices RAI, MTCI, and NDVI were significantly different among five-level HM treatments. Linear regression models predicting chlorophyll concentration (SPAD-502 values) using RIA and MTCI were significant. The new vegetative index RIA possesses similar ability as MTCI in detecting Cu-, Zn-, and Cd-induced stress on wheat at early growth stage. Evidence indicates the potential application of hyperspectral reflectance vegetation indices in detecting the concentration of the heavy trace elements in the wheat canopy is possible.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 41471279].

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