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Original Paper

Fractal based complexity analysis of wheat root system under different heavy metals

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Abstract In this study, fractal geometry was applied to characterize the complexity of the root system morphology of wheat plants under the exposure of heavy metals, namely cadmium (Cd), copper (Cu) and zinc (Zn). We proposed a measure called, relative complexity index (RCI), a ratio based on fractal dimension (FD) before and after exposure to heavy metals. FDs were calculated by box-counting method with digitized and skeletonized images of roots of wheat plants cultivated in hydroculture system. RCI, and relative weight were mesuared under different concentrations of Cd (0.001, 0.01 and 0.05 mM), Cu (0.016, 0.4 and 1.2 mM) and Zn (0.3 and 0.75 mM). Results showed significant reduction of RCI for Cd stress with 0.01 and 0.05, all Cu concentrations and promotion at all zinc concentrations. In comparison, no statistically significant changes were found in conventional relative weight measurement at low concentrations of Cu, Cd and Zn. RCI were more sensitive and were reliable in reflecting the influence of heavy metals than the conventional measure. These results imply that RCI can be an effective measure of the negative and positive effects of heavy metals on the development of complexity of root system under heavy metal exposures.

Key words: complexity, fractal analysis, heavy metals Cd, Cu, Zn, root system, wheat.

Introduction

Plant growth significantly depends on the root system morphology, which includes root length, root branching density, root distribution etc. Roots play a critical role in water and nutriment acquisition under different environment circumstances (Fitter 1987). Extensive studies have focused on methods to determine root growth mainly through length measurement (Chaiwongsar et al. 2012), weight measurement (Xu et al. 2013) and lateral root counting. However, a challenging problem in the measurement of root morphology is quantifying the complexity of the root architecture. If quantification of root complexity could be described, it would provide a strong evidence of existing relationships between root functions and the overall plant performance. Therefore, an accurate method to estimate the complexity of the root system is required.

As a way to estimate the complexity of the root system, a different approach that was based on fractal analysis has been applied (Tatsumi et al. 1989). Fractal is a natural phenomenon or a mathematical set showing a repeating pattern that could be displayed at every scale. Despite considering root systems as fractal objects is still under debate as to the genuinely of having self-similarity (Bernstein 1996) and thus non-integer dimension, application of fractal geometry to describe complexity of roots has found applications in describing the influence of environment (Tatsumi et al. 1989). For instance, effect of different phosphorous conditions on root systems of common bean genotypes by Nielsen (Nielsen et al. 1999), effect of drought stress on rice root system (Wang et al. 2009), and the effect of salt-stressed conditions on corn root system development (Subramanian et al. 2015). To our knowledge up to now, no study has examined the root complexity under heavy metal stress using fractal analysis.

In the last decade, with the development of industry, the environment contamination due to heavy metal has become an increasing global concern as their widespread distribution and toxicity to living things. Heavy metal-induced effects include oxidative stress, genotoxicity, inhibition of the photosynthetic process, and inhibition of root metabolism (Andresen and Küpper 2013). In particular, heavy metal toxicity has been found to affect root formation other than grain yield, seed formation, chlorophyll synthesis, hormone proteins and membrane functions. Cd plays a major role in inhibition of plant growth by accumulation in plant leaf and root (Street et al. 2009). Cd has been shown

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to affect lateral root formation and root development (Ronzan et al. 2018). On the other hand, copper (Cu) plays important role in CO_2 assimilation and ATP synthesis (Yadav 2010). Exposure of plants to excess Cu generates oxidative stress and ROS, and Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al. 2001). Further, excess Cu has a detrimental influence on plants, especially on root growth and morphology (Sheldon and Menzies 2005). Arduini et al. (1994) found that excess Cu can reduce the lateral root index.

Unlike Cd, Zn has been considered as a micronutrient which is needed in small amounts by plant. Under certain optimal concentrations, ZnO (400 nM) showed large root growth of peanut (Prasad et al. 2012). Zn toxicity is apparent as a reduction in the growth of the main root, fewer and shorter lateral roots and a yellowing of roots (Ren et al. 1993). Toxic concentrations of Zn cause inhibition of photosystems I and II and thus a decrease in photosynthesis (Van Assche and Clijsters 1986). The mechanism of the action due to Zn is the displacement of Mg by Zn at the water splitting site in photosystem II (Küpper et al. 1996). Teige et al. (1990) suggested that the primary toxic action of heavy metal is the inhibition of ATP synthesis and therefore energy metabolism in plants. In this study, we have considered the effect of the above three heavy metals, Cd, Cu, and Zn on root system of wheat plant.

Wheat (*Triticum* spp.) is the most widely grown crop in the world and is the first strategic cereal crop for a majority of populations and is the second most important food crop in the developing world after rice (Curtis et al. 2002). It has been known that the heavy metals such as Cd could accumulate in high amounts in the roots of wheat plants (Brunetti et al. 2012).

In this study, to investigate the effects of heavy metals on the wheat root development, we have applied fractal geometry and calculated the fractal dimension (FD) and defined a parameter called relative complexity index (RCI) that is based on FD. FD was calculated using the box-counting method, a popular and easy method used in calculating the fractal dimensions (Tatsumi et al. 1989). Wheat plants were exposed to three Cd concentrations of 0.001, 0.01, and 0.05 mM, three different Cu concentrations of 0.016, 0.4, and 1.2 mM, and two different Zn conditions of 0.3 and 0.75 mM. Under all the conditions, control was the one under 0 mM. The changes in RCI was estimated as a measure of the influence of the heavy metal stress on the root system. For comparison, conventional measures of root weights were also measured and correlation with the RCIs were obtained.

Materials and methods

Plant materials

In this experiment, Norin 61 wheat cultivars were chosen as the plants for study. As samples, we chose equal-sized seedlings one week after germination and photographed every week. In order to avoid root destruction and damage when removing from the soil, the seedlings were grown in a hydroculture system (12 l). For keeping the wheat seedlings under healthy conditions, the plants were watered with nutrient solution (Hyponex, Hyponex Ltd. Japan Corp.) for three times a week. All the plants were grown in a growth chamber (Conviron, Controlled Environmental Led, Winnipeg Manitoba, Canada) under fully controlled environmental conditions of 12 h photoperiod, 27°C day, 20°C night, relative moisture of 65–75% and light intensity of 260–350 μmol m^{-2} s^{-1}.

For Cd exposure growth conditions, root samples were exposed to cadmium chloride solution ($CdCl_2$, Wako Pure Chemical Industries, Ltd., Japan, contain not less than 95%, molecular weight 183.32) for the three concentrations of 0.001, 0.01, and 0.05 mM for over three weeks after germination. 18 replicates were prepared for each concentration. Six replicates were taken weekly for photographing and destructive measurements, such as dry biomass measurement.

Same as Cd exposure experiment, three plant hydroculture systems consisting of 60 samples for Cu experiments were prepared. The concentrations of Cu, as $CuSO_4 \cdot 5H_2O$, (Wako Pure Chemical Industries, Ltd., Japan, containing not less than 95%, molecular weight 249.69) were 0, 0.016, 0.4 and 1.2 mM for over three weeks after germination. Each treatment had six replicates.

For Zn exposure measurement, root samples exposed to different concentrations of 0, 0.3 mM, and 0.75 mM of Zn containing solutions as $Zn(NO_3)_2 \cdot 6H_2O$ (Wako Pure Chemical Industries, Ltd., Japan, containing not less than 95%, molecular weight 297.49) for over three weeks after germination. Again, a total of three plant systems were prepared with one system for control, and the other two under Zn stress with six replicates for each of the three concentrations.

For determining concentrations of the different metals used in the current study, we referred the literatures of Dong et al. (2005) for Cd, Sheldon and Menzies (2005) for Cu, and Prasad and Kundu (1995) for Zn. However, it should be noted that the concentrations of Cu used in the current study were all found to be toxic and thus having negative effect while that of Zn promote growth thus having positive effect.

Traditional measures

The effects of heavy metal exposure were characterized by traditional measure of weights. In order to make weight measurements, at first, the plants (24 plants for Cd and Cu experiment weekly, 18 plants for Zn experiment weekly) along with the root systems were removed carefully from the hydroculture solution. Next, they were washed in distilled water. After washing the roots, the dry weight was determined by at first drying the roots at 105°C for 30 min, followed by keeping them at 70°C for 72 h until the weight became constant. The dry weights of roots and shoots were determined using a SHIMADZU AUX320 analytical balance. We used only dry weights for comparison and all the weights in the following discussions indicate that of dry roots. Relative weight (RW) was defined to evaluate the changes due to exposure to heavy metals. Its definition is as follows:

$$\text{RW\%} = \frac{\text{weight after exposure}}{\text{weight prior exposure}} \times 100$$

Photography, scanning and image processing

Characterization of the complexity of the roots was done by taking photographs of the root systems. To take photographs of the root system, at first, each of the entangled root sample was separated carefully and put in a transparent box. The box was filled with several millimeter depth of water so that the fine roots were clearly displayed. Next, the transparent box was put on a LED backlight panel. Photographs of the entire images of the root systems were taken weekly and digitized (5.72 MB; 6000×4000 pixels) with a digital camera (16.2 megapixels; Nikon D500, Tokyo, Japan). The digitized images were saved in the JPEG format to be analyzed with a PC.

Following the digitization of the images, smoothing was done to remove the spiky noise by applying a median filter (filter size=2), a commonly used filter to remove spiky noise while retaining the edges of the image undisturbed. Next, the images were converted into binary images by setting a threshold which is determined by the illumination system for all the images.

Complexity measures

Next, the binarized images were skeletonized. Skeletonized images were obtained through peeling off as many pixels as possible of the object without affecting the general shape of the binarized object pattern. These steps of image processing, i.e., filtering, binarization and skeletonization were done using custom developed programs using MATLAB (MATLAB R2017b).

With skeletonized images, complexity of the root systems was defined based on fractal dimension (FD). FD was calculated from the skeletonized images using box-counting method (Tatsumi et al. 1989). During estimation of FD, as shown in Figure 1, different scaled grids (r) were applied over the skeletonized image, and the number of boxes that contain the root image were counted. At first, a log–log plot of N(r) against r was obtained followed by a linear regression fitting to calculate the slope D or the fractal dimension FD of the image, as expressed by, $\text{FD}=\log \text{N}(r)/\log(1/r)$.

The FD takes unity for a simple line object and increases towards 2 as the complexity of the line object increases, ($1\leq \text{FD}<2$). Therefore, FD being one would mean a simple root having one dimension or a line and a value larger than one would indicate the increasing complexity of the root system. Thus, FD indicates the degree of complexity of the root system and depending on the exposure to heavy metals, FD is expected to decrease or the root system getting less complex. Accordingly, we can define a complexity index (CI) as;

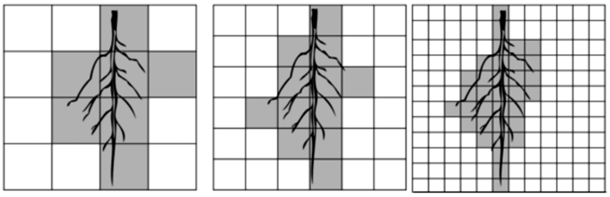


Figure 1. Different sized grids (r) were applied over the skeletonized images, and the number of boxes that contain the root image were counted for estimating the FD of root system.

$$\text{CI} = \text{FD} - 1$$

The complexity index of a line is 0, and the complexity index for the most complex root structure that approaches to area rather than a line is 1 and percentage of relative complexity index as:

$$\text{RCI} = \frac{\text{CI}_{\text{Post}}}{\text{CI}_{\text{Pre}}} \times 100$$

where CI_{Pre} and CI_{Post} are the complexity indices before and after heavy metal exposure, respectively.

Statistics

RCI and plant development related weight parameter were statically analyzed using ORIGIN software (Origin Pro 8J). Statistical significance was assessed by hypothesis testing at 5% level with a Two-sample *t*-test analyzed using ORIGIN software (Origin Pro 8J).

Results

We calculated the change in complexity index under the exposure of different heavy metals Cd, Cu, and Zn and compared that with the corresponding changes in the root dry weights.

Relative change in complexity as percentage

Figures 2–4 show the skeleton images of root architecture obtained under the respective exposure of Cd, Cu, and Zn for three weeks and at different concentrations. In all the three cases, as can be seen, in comparison to the control, there are differences in the development of the root system. Under the exposure of Cd and Cu, as seen from the skeletonized images, the root system has less lateral branching, and the number of roots or the density of roots appear to be less. These changes are pronounced with increasing concentrations of Cd and Cu and at higher concentrations, the overall root length appear to be shorter.

In contrast, for exposure under Zn, there is increased root length as well as increased root branching and there appear to be more number of roots or there is increased root density. These changes are increasingly evident

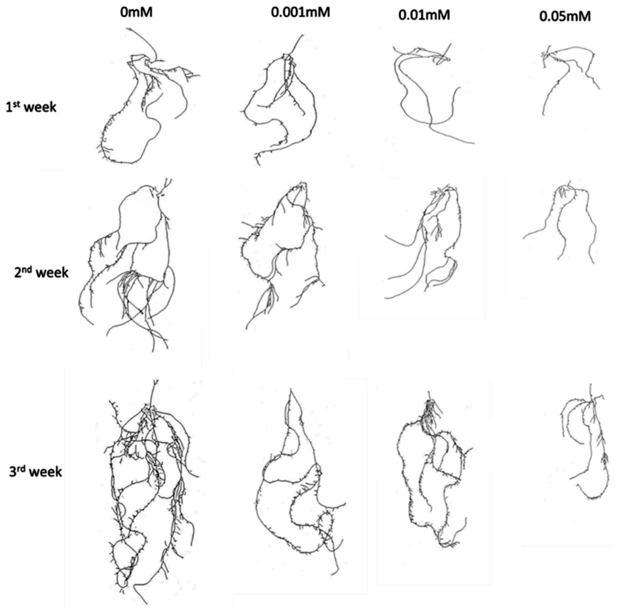


Figure 2. Skeleton images of wheat root under four Cd concentrations, 0, 0.001, 0.01 and 0.05 mM obtained for three consecutive weeks. In comparison to control of 0 mM, under the exposure of Cd, the skeletonized images showed less lateral branching, and decreased number of roots.

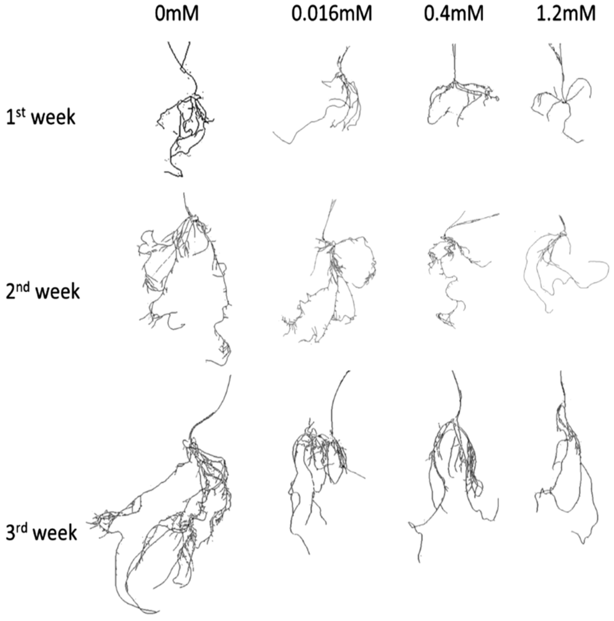


Figure 3. Skeleton images of wheat root under four Cu concentrations, 0, 0.016, 0.4, and 1.2 mM obtained for three consecutive weeks. In comparison to control of 0 mM, under the exposure of Cu, the skeletonized images showed less lateral branching, and decreased number of roots.

with increasing concentrations and also with increasing exposure weeks.

However, except for the qualitative differences, it is difficult to assess the quantitative changes in the differences with increasing exposures of all the heavy metals Cd, Cu, and Zn. Thus, there is a need for a measure to assess the difference quantitatively and is done using change in relative complexity index (RCI) which is based on FD that is described in subsection of complexity measures. Results of FD obtained over three weeks under different concentrations of Cd, Cu, and Zn are shown in Tables 1–3 for each week.

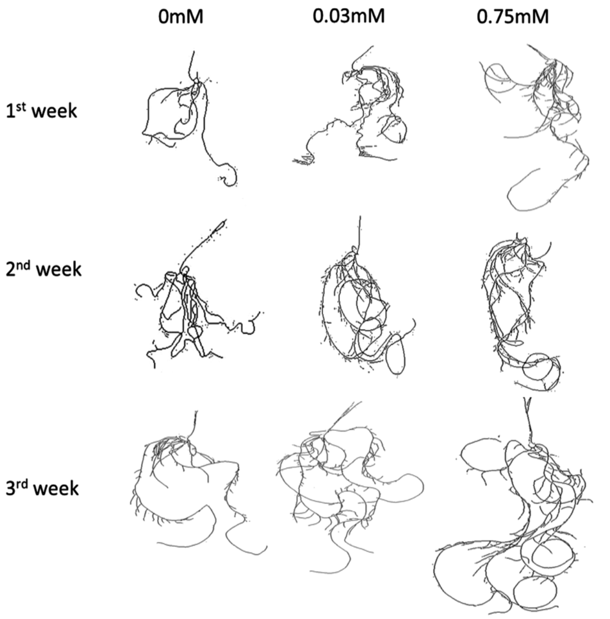


Figure 4. Skeleton images of wheat root under three Zn concentrations, 0, 0.03, and 0.75 mM obtained for three consecutive weeks. In comparison to control of 0 mM, under the exposure Zn, the skeletonized images showed increased lateral branching and root density while in contrast for Cd and Cu, there is decreased lateral branching and root density.

Figure 5(a)–(c) show the results of RCI under the exposure of Cd, Cu and Zn, respectively as a function of exposure weeks for different concentrations. As seen from the Figure 5(a) and (b), the values of RCI for control shown in black square was the highest. Further, as seen, compared with the control, there were clear reductions in RCI under the presence of Cd and Cu with increasing concentrations as shown by different symbols and the reductions are larger with increasing exposure weeks.

Effect of different Cd concentration of on RCI was given in Figure 5(a). The RCI was maximum in control condition for each week. The significant reduction in RCIs can be seen even within the first week, that for the lowest concentration of 0.001 mM and the reduction percentage was 12.5%. For second and third weeks, the reduction percentages compared with control were found to be respectively, 12.4% and 10.7% for 0.001 mM concentration. The highest reduction in RCI compared to control is for Cd concentration of 0.05 mM, and the reduction percentages for each week were 50.0%, 62.5%, and 64.3%, respectively. Therefore, the highly toxic effects of Cd can be clearly seen through the measure of

Table 1. FD for wheat root skeleton images under four different Cd concentrations obtained over a three week period.

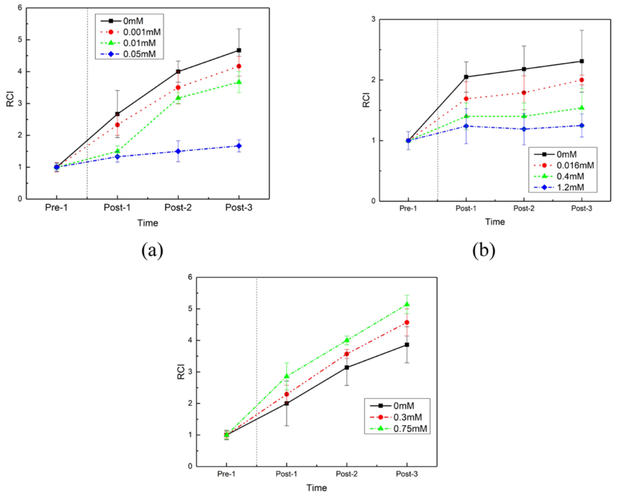
	0 mM	0.001 mM	0.01 mM	0.05 mM
1st week	1.161	1.140	1.091	1.083
2nd week	1.243	1.213	1.191	1.092
3rd week	1.282	1.251	1.222	1.134

Table 2. FD for wheat root skeleton images under four different Cu concentrations obtained over a three week period.

	0 mM	0.016 mM	0.4 mM	1.2 mM
1st week	1.205	1.169	1.140	1.122
2nd week	1.218	1.179	1.141	1.124
3rd week	1.231	1.200	1.154	1.125

Table 3. FD for wheat root skeleton images under three different Zn concentrations obtained over a three week period.

	0 mM	0.3 mM	0.75 mM
1st week	1.142	1.169	1.201
2nd week	1.220	1.249	1.284
3rd week	1.271	1.318	1.362



(a) (b)

(c)

Figure 5. Relative Complex Indices (RCI) in percentage as a function of time under (a) four Cd concentrations, 0, 0.001, 0.01 and 0.05 mM, (b) four Cu concentrations, 0, 0.016, 0.4 and 1.2 mM, and (c) three Zn concentrations, 0, 0.03, and 0.75 mM. Data are means±SD (*n*=6).

RCI even for small concentrations of 0.001 mM of Cd. Statistical analysis showed significant difference ($p<0.05$) between the control and all of the RCIs under all Cd concentrations.

Similar harmful effects of Cu can be seen in Figure 5(b). The lowest RCI was obtained for the highest Cu concentration of 1.2 mM and the highest value for control condition under each week. For the first week, RCI values ranged from 2.05 for control to 1.19 for 1.2 mM concentration of Cu indicating that the changes were large even at an early stage. However, with increasing weeks, the pronounced effects of Cu were clearly seen. The RCI value of over 2.18 for control drastically decreased to around 1.79 for 0.016 mM with distinct reductions seen for other Cu concentrations also. Again, a similar reduction tendency can be seen for third week for all Cu concentrations. The reduction percentages for third week of RCI were found to be respectively, 14%, 34%, and 46% for Cu concentrations with 0.016, 0.4, and 1.2 mM, respectively. Statistical analysis showed significant difference ($p<0.05$) between the control and all of the RCIs under all Cu concentrations.

Contrary to the results of Cd and Cu, the promotion effect on lateral root formation could be seen clearly form the skeletonized images (Figure 5(c)) after Zn exposure. Figure 5(c) indicates the RCI under the different Zn concentrations, 0, 0.3, and 0.75 mM. RCI clearly increased over time after exposure to both of the concentrations, 0.3 and 0.75 mM. Under 0.3 mM, significant increments of 14.3%, 13.6%, and 18.5% could be observed over three weeks. For higher concentration of 0.75 mM, the increase was larger with 42.9%, 27.3%, and 33.3% for first to three weeks.

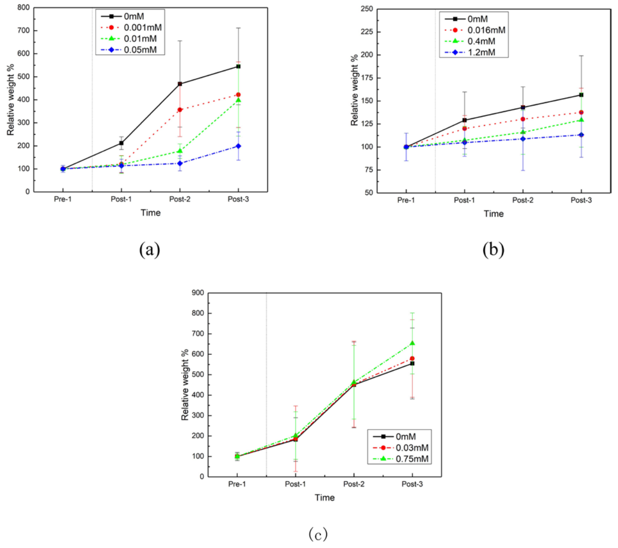
Statistical analysis showed significant difference ($p<0.05$) between the control and all of the FD under 0.3 and 0.75 mM Zn stress over the whole duration of the experiment.

Relative weight change

Relative weight change is estimated by normalizing the weight of Cd exposed wheat plants with that of the unexposed ones of first one week. The effect of Cd on root relative dry weight of wheat varied with four concentrations are shown in Figure 6(a). The addition of Cd 0.001, 0.01, and 0.05 mM, led to a decrease in relative root weight in comparison to those of the control can be seen with increasing concentrations of Cd for wheat.

For each week, the largest relative root biomass was observed under control condition, and the lowest relative root biomass was observed under highest Cd concentration (0.05 mM). But significant changes could not be found under lower Cd concentrations of 0.001 and 0.01 mM on whole duration of the experiment. The significant reductions were found to be respectively, 73.5% and 63.5% for 0.05 mM at post-two and post-three week.

The relative weight results after three weeks of experiments indicated that all the Cu treatments of 0.016, 0.04, and 1.2 mM reduced the root weight than control and increasing Cu treatments reduced relative dry weights of roots (shown in Figure 6(b)). Compared to the control, application of 0.016 mM Cu reduced relative weight by 16.9%, 18.8%, and 17.4% for each week. At the highest level of Cu stress (1.2 mM), the reduction in relative weight for 3 weeks of post-treatment showed about 18.9%, 23.9%, and 27.7% of controls, respectively.



(a)

(b)

(c)

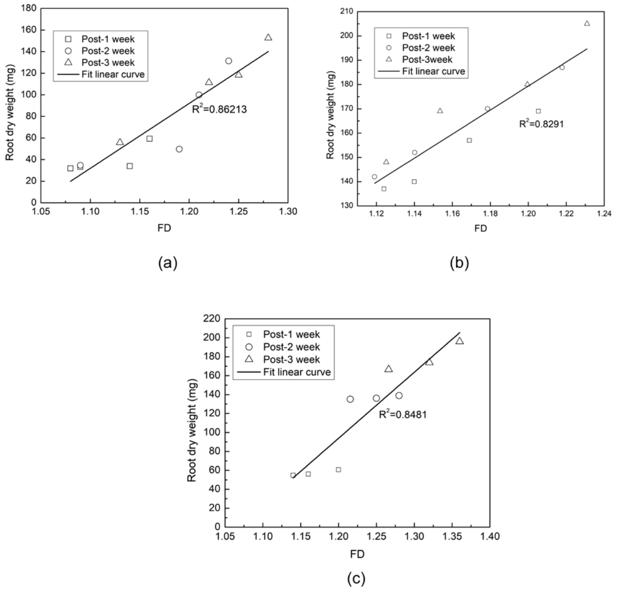
Figure 6. Relative root dry weight in percentage as a function of time under (a) four Cd concentrations, 0, 0.001, 0.01 and 0.05 mM, (b) four Cu concentrations, 0, 0.016, 0.4 and 1.2 mM, and (c) three Zn concentrations, 0, 0.03, and 0.75 mM. Data are means±SD ($n=6$).

Results of relative weight changes of wheat under Zn exposure over three weeks are shown in Figure 6(c). Under all the concentrations of Zn, increment of relative biomass was almost the same for the first and second weeks. Compared with the results for Cd and Cu, the largest root weight was observed under highest Zn concentration of 0.75 mM, and the lowest relative biomass was observed under the control condition. Increment of root biomass under 0.3 and 0.75 mM were respectively, 4.4% and 14.7% at post-3 week. However, these differences were not significant under all Zn concentrations compared with the control for all three weeks. The small increase in root relative weight suggested for possible stimulation of root growth under the exposure of Zn while for three weeks exposure of Cd and Cu, there are inhibitions of root growth.

Discussion

The present study explored the positive and negative effects under Zn and Cd, Cu treatments on roots of wheat plants by using fractal dimensions as complexity measure and compared with conventional measures of root weight.

Cd stress was investigated under three different concentrations of 0.001 mM, 0.01 mM, and 0.05 mM. The root growth inhibition was observed under all of the Cd concentrations. Similar results were also found in the wheat under Cd stress by Eker et al. which is directly related to nutrient uptake in root and shoot (Eker et al. 2013). Tai et al. referred that Cd stress significantly caused the adverse effects on plant growth and root morphology of Switch grass seedlings (Tai et al. 2017).



(a)

(b)

(c)

Figure 7. Correlation between fractal dimensions and root dry weight under (a) Cd, (b) Cu, (c) Zn. Data are means±SD ($n=6$). The straight lines indicate the fitting of the correlation and as can be seen there exists strong correlation between the measures of FD and the relative dry weight with the correlaiton coefficients larger than 0.8.

In our investigation, Relative Complexity Index or RCI was found to be sensitive enough that it showed significant reduction ($p<0.05$) even under concentration of 0.01 mM or 0.05 mM in comparison to the relative weight. Our measure is found to be sensitive enough compared to other study which used 50 mM of Cd for 15 days exposure (Rodríguez-Serrano et al. 2006).

Kubo et al. (2011) also reported similar results that root branching slowly or limited development was related by lower Cd uptake at seedling stage of Japanese wheat. Lux et al. (2010) researched the root structure of plant *Merwilla plumbea* under Cd stress and described the response of this plant under Cd stress through Cd uptake in different organs and growth parameters. Their results showed that the roots of plant exposed to Cd were significantly stunted and root formation was inhibited (Lux et al. 2011), which is similar to the observation done in this study. The reason for growth inhibition under Cd stress was mainly due to increasing Cd concentration in plant tissues resulting in decrease of chlorophyll content and thus photosynthesis rate (Miller et al. 2016; Paunov et al. 2018).

The root biomass was found to be highly correlated with the RCI and thus fractal dimension values in Figure 7(a) for high concentrations of Cd but not at lower concentrations whereas RCI was found to be more sensitive. As Cd concentrations increased, root biomass and FD reduced with each week. FD showed a positive relationship with conventional measurements of root dry weight ($R^2=0.8621$) under a Cd concentration of 0.05 mM.

Copper (Cu) is an essential micronutrient for plant growth, and the normal range in the growing medium is from 0.05 to 0.5 mg/kg, while in plant tissues usually ranges from 3 to 10 mg/kg (Carruthers 2016). However, excess Cu has a detrimental influence on plants, especially on root growth and morphology (Sheldon and Menzies 2005). Cu tends to accumulate mainly in the root tissue (Marschner 1995), and its toxicity is mainly on root growth. Cu toxicity damages to plant roots with symptoms including disruption of the root cuticle, reduced root hair proliferation and severe deformation of root structure. It is estimated that the critical concentration of Cu in the nutrient solution associated with a 10% reduction in plant growth is from 0.6 and 1.1 μM. Excess copper in the growing medium can also restrict root growth by burning the root tips. High levels of copper can compete with plant uptake of iron and zinc (Sheldon and Menzies 2005). In our experiments, significant reductions in RCI ($p<0.05$) between the control and all Cu concentrations of 0.016, 0.4 and 1.2 mM stress were found. Further, at high concentrations, FD (Figure 7(b)) also showed a positive relationship with root dry weight ($R^2=0.8291$). High concentration of Cu causes a reduction in plasma membrane integrity in plant roots, which is the mechanism by which Cu toxicity retards root growth (Arduini et al. 1995; Luna et al. 1994).

In addition, zinc is a plant micronutrient for plant growth which is involved in many physiological functions including auxins formation, chlorophyll formation and protein synthesis. Our results showed, as Zn concentrations increased, the complexity of root structure increased with growth weight getting larger. FD also showed a positive relationship with root dry weight ($R^2=0.8481$) (Figure 7(c)). For all the cases, as weight developed with increasing age, the complexity of root increased. From the increments in RW after exposure to Zn, we found the positive effect for both concentrations of 0.3 and 0.75 mM. A number of researchers have reported the essentiality and the role of zinc for plant growth and yield (Lucini and Bernardo 2015; Kisan et al. 2015). Efremova et al. mentioned that zinc compounds had beneficial effect on dry biomass of *Saccharomyces cerevisiae* CNMN-Y-11. Compared with control, the yeast biomass obtained the maximum with increasement rate of 28–38% after utilization of zinc compounds (Efremova et al. 2013). Yilmaz et al. also found that Zn treatment could lead to increases in grain yield and biomass production with 260% compare with control (Yilmaz et al. 1998). However, considering our results, the significant difference could not be shown in the increments of dry weight.

The different Zn treatments affected the complexity of root system, as the overall mean RCI value for the whole root system was significantly increased. In other words, the results of fractal geometry based analysis suggested Zn application promoted lateral root formation. Nair and Chung studied the 20 mg/l zinc oxide nanoparticles treatment triggered a 9% increase in lateral root formation in *Arabidopsis thaliana* seedlings. (Nair and Chung 2017) Our results using fractal geometry as a measure for the complexity of root system suggest that the roots were affected from an earlier stage of heavy metal (Cd, Cu and Zn) exposure and start to accumulate more heavy metals in themselves than the other organs. Compared to the conventional measure (dry weight), RCI could be used as a more sensitive measure to evaluate the positive and negative effects of heavy metal on the root system of wheat plants.

Conclusion

In this study, the fractal geometry was used to evaluate the root architecture of wheat under heavy metals of Cd, Cu and Zn exposure for a period of three weeks. Root systems were photographed, binarized and skeletonized to determine the fractal dimension. Fractal dimension was used to estimate the complexity of the system. The results imply that a fractal based complexity measure could be sensitive enough that there could be significant decrease in the complexity of the root system even under smaller concentration of Cd and Cu in comparison to the traditional measures of root weight. Simultaneously, the positive effect of the micronutrient could also be assessed by the complexity measure RCI in comparison to the weight which failed to show any significant differences even at high concentrations. Fractal based approach to analyze the effects of heavy metals on root architecture can be an effective measure for the structural development of the root system.

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