Hyperspectral reflectance features of water hyacinth growing under feeding stresses of Neochetina spp. and different heavy metal pollutants.

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Abstract

Spectral signatures of water hyacinth grown with biocontrol agents (*Neochetina eichornia* and *N. bruchi*) and various heavy metal pollutants were collected at the plant canopy level using a hand-held spectrometer to detect biocontrol agent and heavy metal-induced plant stresses and the interaction between the two stressors. Water hyacinth was grown in 65L tubs, each with a single treatment, from one of; As (1 mg/L), Au (1 mg/L), Cu (2 mg/L), Fe (0.5, 2 and 4 mg/L), Hg (1 mg/L), Mn (0.5, 2 and 4 mg/L), U (1 mg/L) and Zn (4 mg/L), with the exception of the control treatment. Spectral measurements were taken before and after the addition of the weevils. Several spectral indicators of plant stress including RE_NDVI, $mNDVI_{705}$, mSR, PRI, red-edge position (REP) calculated using first derivative and linear extrapolation and WBI (to detect water stress) were used to identify biotic and abiotic induced plant stresses of water hyacinth. The spectral indicators of both metal and weevil plant stressors were correlated with the leaf chlorophyll content from the SPAD readings at the end of the experiment. Correlation results of $mNDVI_{705}$ was the highest followed by REP_LE. Cu-, Hg-, and Zn-treated plants showed significantly lower chlorophyll contents compared with the control treatment. A similar trend with four additional treatments (As, Fe-M, Mn-L and Mn-H) was seen after the release of the weevils, indicating plant stress due to feeding by the biocontrol agent. These results indicate that hyperspectral remote sensing has potential as a tool to determine the health status of water hyacinth from a remote location, to inform management interventions in the control of the weed. However, its usage at a larger scape requires further studies.

1. Introduction

Plants growing in metalliferous soils and most aquatic macrophytes have an enormous capacity to accumulate heavy metals in their plant tissues. In the last two decades efficient accumulator plants have been targeted for phytoremediation of land and water pollution caused by anthropogenic activities and have proven to be viable for remediating contaminated environments in many wetlands and industrial areas (Eapen and D'Souza, 2005). For instance, water hyacinth has been used in a constructed wetland in Taiwan to remove large amounts of lead, copper and zinc (Liao and Chang, 2004). Therefore, wetlands that are invaded by water hyacinth could be regarded as "nature's kidneys" that purify polluted water (Malik, 2007). Nevertheless, the accumulation and storage of heavy metals in plant tissues is known to influence insect herbivory and this may pose a problem to management of invasive alien weeds with biocontrol agents (Boyd, 2010). Plants growing in metalliferous sites have reduced biotic stresses compared to the same plants growing in unpolluted soils. Noret *et al*., (2006) indicated that only one out of the total 63 different types of insect herbivores that are known to feed on *Silene vulgaris* Garcke (Caryophyllaceae) was actually found to attack this plant when grown on contaminated sites.

Water hyacinth is an invasive aquatic plant causing extensive environmental problems in South Africa (Coetzee *et al*., 2011). Extensive water hyacinth infestation causes several economic and environmental problems. For example, water hyacinth prevents light penetration and reduces water oxygen levels. It also obstructs fishing, irrigation, navigation, recreational activities and impedes power generation by obstructing turbines (Malik, 2007). The massive growth of water hyacinth biomass above and below water is usually responsible for gradually terminating the lives of streams and rivers by choking them to death (Tiwari *et al*., 2007).

There are six water hyacinth biocontrol agents introduced from Latin America and established successfully in South Africa. Among these agents, the water hyacinth weevils *N. eichhorniae* and *N. bruchi* are widely used in the country (Cilliers and Neser, 1991). These nocturnal weevils are about 4-5 mm long and spend the day sheltering in the leaf sheath or inside rolled leaves (Oberholzer, 2001). On average, the female produces 350 to 400 eggs in its life span and these are laid either deep on the younger leaf tissue or on the upper surface of older petioles for *N. eichhorniae* and *N. Bruchi,* respectively (Oberholzer, 2001). When the eggs hatch in about two weeks time, the larvae start feeding by mining and tunnelling into the petiole towards the crown, while the adult weevils feed on leaves, usually leaving behind characteristic feeding scars (Del Fosse et al., 1976). Ajuonu *et al*. (2007) measured a maximum of 212 scars per leaf, caused by feeding of weevils and the damage caused by *N. bruchi* was twice that of *N. eichhorniae*. Both weevils can cause considerable damage to water hyacinth but have not satisfactorily controlled the plant yet.

Despite several attempts at biocontrol, water hyacinth in South Africa remains a problem, encouraging new approaches to integrated control methods. Eutrophication and water pollution in South Africa are among many factors that are commonly linked with the extensive growth of water hyacinth, making biocontrol more difficult (Coetzee and Hill, 2012). Currently the paradigm of water hyacinth control in South has shifted to an integrated management that combines biocontrol with herbicide method (Byrne *et al*., 2010). However, to facilitate the decisions of implementing a time-efficient measure, an efficient method of data collection is required. In recent years hyperspectral sensors, at a small scale using a hand held spectrometer or at a larger scale using images from airborne and satellite platforms, have emerged as a powerful tool for monitoring plant health status and monitoring the encroachment of different alien invasive plants in different habitats.

1.1 Spectral indicators of plant health status

Most plants with healthy green leaves absorb highly in the blue (400-500 nm) and red (600-700 nm) regions of the electromagnetic spectrum. Healthy plants also reflect highly in the green region (500-600 nm) and beyond the visible range between (700-1300 nm) of the light spectrum **(**Mirik *et al*., 2007). Furthermore, leaf chlorophyll-a and chlorophyll-b accounts for the red leaf fluorescence in the 600-700 nm range (Liew *et al*., 2008). Chlorophyll fluorescence is the light re-emitted by chlorophyll molecules of plant leaves after absorption as opposed to light reflectance which is the amount of incident light directly reflected back from the surface. Both the internal leaf structures and the leaf pigments are directly influenced by the physiological status of the plant, hence any alteration as a result of stressors will change the spectral signature of the vegetation (Blackburn, 1998) and this provides information on the plant health status (Peñuelas and Filella, 1998; Mirik *et al*., 2007).

Water deficiency, pests, pathogens, frost and soil and water pollution are among some of the environmental factors that prevent plant chlorophyll development, thus affecting the spectral signature of vegetations. For instance, spectral absorption of light in the red band decreases when photosynthetic activities are impaired due to a reduction in the total chlorophyll concentration and a decrease in the chlorophyll to carotenoid ratio (Rock *et al*., 1988). Such stress-induced variation in chlorophyll and other pigments increases chlorophyll fluorescence in the red band as a result of the dissipated excess light energy accumulated by the chlorophyll molecule, which in turn exceeds the limit of the declining photosynthetic activity, to protect potential damage in the chloroplast (Liew *et al*., 2008; Marlin, *et al* in press). Variations in leaf chlorophyll cause shifts in the red-near infrared slope known as the red-edge slope and also the inflection point of the red-edge slope known as red-edge point (REP) (Curran *et al*., 1990; Smith *et al*., 2004). This slope occurs between the maximum point of chlorophyll absorption in the red band (around 680 nm) and around 780 nm where the highest spectral reflectance in plants is observed due to increased multiple scattering of radiation in the intercellular spaces of the leaf mesophyll (Smith *et al*., 2004). A slight shift in the red-edge slope or REP towards the shorter wavelength ("blue shift") is observed when green plants are under stress conditions such as those induced by heavy metal concentrations, (Rock *et al*., 1988; Carter *et. al*., 1993). The REP shifts towards the longer wavelength ("red shift") with increasing chlorophyll concentration (Liew *et al*., 2008).

Different studies have applied shifts in the REP at the region of 680-780 nm as a significant indicator of plant stress. This is because the REP is only slightly influenced by factors such as trichome density, variation in leaf structure (Liew *et al*., 2008). The slope of the red edge changes as a healthy and actively photosynthesizing plant faces different stress levels. For instance Rock *et al*. (1988) indicated that a 5 nm blue shift of the red edge position was detected in spruce specimens collected from sites of high air pollution damage, and in a western conifer plants exposed to O_3, SO_2 pollution and acid misting in a controlled environment.

1.2.The impact of heavy metal pollution on plants

The accumulation of heavy metals in plant tissues disrupts physiological activities through oxidative tissue damage since the uptake of elemental metals can produce increased amounts of toxic oxygen by inhibiting both plant photosystems (photosystem I and II) (Navari-Izzo *et al*., 1998). For instance Maksymiec *et al*., (1994) showed the photoinhibitory damage caused by excess Cu to PSII, when it reaches the leaf tissues. Excess reactive oxygen species generated by such metals in the plant's shoots, exposes cells to reactive free radicals that catalyse the peroxidation of unsaturated fatty acids, membrane dismantling, ion leakage and DNA-strand cleavage among others (Rascio and Navari-Izzo, 2011). Most plants growing under the stress of such heavy metals can survive by either preventing the metals from entering into the root cells through exuded organic acids or anionic substances within the cell walls to which the metal ions are bound in the non-protoplasmic plant features such as the cell wall and intercellular materials (Rascio and Navari-Izzo, 2011). Most heavy metal contaminants are accumulated in the roots of water hyacinth rather than in the shoot system (Malik, 2007), except for some which are transported in to the upper biomass of the plant such as Se (Table 1) (Zhu *et al*., 1999). For instance a linear correlation of metal accumulation was found in the order of roots>stems>leaves of water hyacinth with increase of contaminant solution concentration (Stratford *et al*., 1984), as was the case with Cr, Cu, Ni and As (Lu *et al*., 2004). The fact that water hyacinth and many other aquatic plants store most of the heavy metals in their root system may suggest why these plants are usually resistant to heavy metals and are used as good candidates of phytoremediation.

The information on the heavy metals interaction with biocontrol agents on water hyacinth weed is not well established. Despite the weed being one of the worst aquatic invaders, the hyperspectral analysis of water hyacinth in the past is nothing more than general mapping of plant coverage (Cavilli *et al*., 2009; Hestir *et al*., 2008; Underwood *et al*., 2006; Everitt, *et al*., 1999).

In this study we investigated the water hyacinth plant health status using a hand-held spectrometer, the Analytical Spectral Device (ASD). The ASD is limited to plant-scale monitoring of plant stresses. However, it has better temporal, spectral, and spatial resolution than airborne data. Some existing hyperspectral indicators were used to determine both biotic and abiotic plant stressors and their interactions between them.

2 Material and Methods

2.1. Experimental design

2.2. Spectral measurement

2.3. Data analysis

A single-component system trial of water hyacinth grown under different biotic and abiotic conditions was conducted in 65 L tubs in a “greenhouse tent” at the University of Witwatersrand, Johannesburg (South Africa). Tubs were first conditioned with sulphuric acid (pH 1.5) for a week. The acidic water was neutralized with sodium hydroxide (NaOH) and disposed of. The tubs were thoroughly washed with tap water, rinsed and dried. Plants of water hyacinth were grown with a single heavy metal treatment in each tub under a clear non-UV screening greenhouse plastic tent (UVA-clear 200MIC, supplied by Vegtech 2000, Cape Town, South Africa) at the roof of the OLS building of the University. The trials were conducted for a period of 60 days starting in late spring of 2011 and ending in early summer of 2012, with minimum, maximum, and average air temperatures inside the plastic tent being 6°C, 42°C and 24°C respectively.

The water hyacinth used in the tub experiments was transplanted from a pond at the University of the Witwatersrand and were originally obtained from Delta Park, Johannesburg two years prior to the experiment. Water hyacinth plants were added to the tubs and pools at same phenostage (short, green and healthy, “bulbous” stage) and were left to grow between two to three weeks, before the start of the trials.

Spectral measurements of water hyacinth were taken from plant canopy at a height of 80 cm above the top of the plants, using a hand-held spectrometer manufactured by the Analytical Spectral DevicesT" (ASD) (Boulder, Colorado, USA), with a 25° Field of View (FOV) through a permanent fibre optic cable. This device has a narrow band 1 nm sampling interval and acquires spectral data between 350-2500 nm. All spectral measurements were taken during a warm day with a clear sky between 10:00 in the morning to 14:00 in the afternoon.

After the last spectral measurement at the end of the experiment, leaf chlorophyll measurements of the plants were also taken with leaf chlorophyll meter (SPAD-502 Minolta, Japan) for comparison and interpretation of the spectral signature from the ASD. SPAD-502 readings were taken on ten leaf samples from each replicate of each treatment in the tub totalling 30 leaf SPAD-502 readings per treatment. The larval and adult leaf damages of the water hyacinth weevil were also counted as larval mining and adult feeding scars, respectively to compare results of the spectral data.

2.2 Experimental set up

A total of 39 tubs for 13 different treatments, with each treatment having three replicates were arranged randomly in four rows (Fig. 1). Tubs were filled with about 45 litres of tap water, and a ¼ of the complete strength of Hoagland solutions was added to each tub using a plastic syringe and stirred thoroughly with a plastic rod., Each tub was equipped with a submersible fish tank pump (flow rate 400 litres/hr model PH400; power head pump) to agitate all treatments.

Ten short, green and healthy water hyacinth plants of the same size were washed and rinsed several times with tap water and then added to each tub, left to grow for a week. All metal treatments were added to each tub in the same way as the Hoagland solution, except that the plants were first raised above the water before adding the treatments, to facilitate the stirring process. Metals added were As, Au, Cu, Fe, Hg, Mn, U and Zn, from which Fe and Mn were also used as treatments to test the dose response of the plants as low, medium and high concentrations (Table 1).

Water loss from each tub due to evapo-transpiration was compensated by topping up each tub every four to six days. The experiment was conducted in two phases for about 60 days. The first 18 days (metal uptake phase) were used to investigate the spectral signature of water hyacinth as a result of heavy metal impacts, after which 60 water hyacinth weevils (an average of 3.5 weevils per plant) from both *Neochetina eichhorniae* and *N. bruchi*) were added to each tub for the second phase (the biocontrol or weevil treatment phase). Spectral measurements were taken before (week-3) and after (week-9) the addition of the weevils. Each treatment had three replicates and the spectral measurement on each replica was repeated three times giving a total of nine spectral data for each treatment at each sampling occasion.

2.3 Spectral analysis

Different indices were implemented to analyse the spectral data from the ASD (Table 2). The first derivative spectra were calculated using the first-difference approach, which computes the difference between adjacent wavebands (Dawson and Curran, 1998). The REP was computed using the linear extrapolation method proposed by Cho and Skidmore, (2006). Shift in the REP before and after the application of water hyacinth weevils was calculated by subtracting the wavelengths of each heavy metal treatment in week-3 (the metal uptake phase) and week-9 from the respective control treatments. One-way analysis of variance (ANOVA) followed by Fisher's Least Significant difference (LSD) post hoc test was conducted to evaluate whether significant differences exist between the chlorophyll content of the water hyacinth measured or calculated under different treatments. Regression analysis was used to assess the relationships between the SPAD-502 reading of leaf chlorophyll content and the spectral stress indicators (spectral indices).

3 Results

After three weeks with a single heavy metal, each treatment showed a remarkable variation in light absorption and reflectance of water. The control showed the highest absorption at around

670 nm followed by U and Mn-H treatments (Fig. 2A). Cu, Zn and Hg treatments showed the least absorption. A similar trend was also seen indicated in the near infrared (NIR) region where U, Mn-H and control treatments had a higher reflectance than Cu, Zn and Hg which caused the lowest reflectance (Fig. 2A). Six weeks after the addition of biocontrol agents (week-9), the spectral reflectance of each treatment was even more distinct (Fig. 2B). The spectral absorption and reflectance features at the VNIR decreased after the addition f the weevils (Fig. 2A and 2B). Absorption at the red band was highest for the Fe-H treatment, followed by the control, while Cu, Zn and Hg treatments were the lowest (Fig. 2B). The highest reflectance was showed in Fe-L followed by the control treatment while the Cu, Fe-M and Hg treatments had the lowest reflectance (Fig. 2B).

When the red edge first derivative curves for only the treatments that were significantly different from the control were computed, two distinctly defined peaks were observed (Fig. 3A and B). These two characteristic peaks in the red edge, along with the shift of the red edge position to either the shorter (the blue shift) or the longer wavelengths are associated with plant health status, and these features were more clearly distinguishable in the treatments after the weevils than before the addition of the weevils. The same elements (Cu, Zn and Hg) showed an increase in the first peak at around 702 nm and decrease in the second at ~ 718 nm, opposite to the control treatment (Fig. 3A). Similarly, the Cu, Zn treatments followed by Mn-L and Mn-M treatments showed the highest first peak while the control treatment had the highest second peak, after the addition of the weevils (Fig. 3B).

The canopy chlorophyll content of both trials, before and after addition of weevils, calculated using the modified red edge index, $mNDVI_{705}$ showed significant differences between treatments (($F12, 101) = 17.206$, $p < 0.001$) and $F(12, 101) = 18.6235$, $p < 001$) respectively) (Fig. 4A and B). In the first three weeks of the trial (week-3) Copper, Hg and Zn treatments were significantly lower from the control and all the other treatments. In the second phase of the trial (After the addition of the weevils) $mNDVI_{705}$ generally had dropped significantly ($F12, 25) = 4.4996$, $p < 0.001$) and the same three treatments (Cu, Hg and Zn) were significantly lower from the control treatment, but not from the rest of the other treatments in the trial. The spectral index, $mNDVI_{705}$ in both trials (before and after the addition of the weevils), of iron and manganese dose response treatments, showed no significant difference with the exception of Fe-M which was significantly lower than the Fe-H treatment (Fig 4A and B).

The results of the red-edge position (REP), calculated from the first derivative spectra using the linear REP_LE technique showed significant differences between treatments before and after the addition of the weevils ($F_{(12,\ 25)}$=3.1187, P<0.008) and ($F_{(12,\ 25)}$=3.7748, P<0.003), respectively) (Fig. 4C and D). The general trend of the results calculated with REP_LE followed the same pattern as those in the $mNDVI_{705}$ results in both trials (before and after addition of the weevils). The treatments of Cu, Hg and Zn consistently revealed a significant difference from all the other treatments in the first three weeks (Fig. 4C). The REP significantly decreased in the second phase (week-9) ($F(12, 25) = 3.9958$, p<001) and Cu remained significantly different from all the rest of the treatments (Fig. 4D). The iron and manganese dose response treatments showed no significant difference between them in both trials (week-3 and week-9). The REP of Cu, Hg and Zn treatments showed the highest blue shift of approximately ~ 5.5 nm from the control, compared to all the other treatments (Fig. 4C). The blue shifts increased an additional 14.5, 2.5 and 1.5 nanometres for the Cu, Hg and Zn treatments respectively when the weevils were added (Fig. 4D).

The water band index, of the metal and weevil phase trials showed significant difference between treatments ((F12, 101) = 11.3062, $p < 0.001$, and $F(12, 101) = 4.9604$, p = 001) respectively) (Fig. 5A and B). In the first three weeks of the metal phase trial Cu and Hg showed significantly the lowest canopy water content (CWC) followed by Zn which was not significantly different from the Fe-L treatment (Fig. 5A). The pattern of the plant water status in the second phase of the trial after the addition of the weevils however, was opposite of the results in the week-3 and decreased significantly (F12, 25) = 2.795, $p < 0.015$). The U, Fe-H and Au which were among some of those with significantly higher CWC compared to Cu, Hg and Zn treatments in the first phase of the metal trial, showed significantly lower CWC in the second phase of the weevil trial compared to the same elements with the exception of Zn treatment (Fig 5B). The pattern of iron and manganese dose response treatment was similar to that of the mNDVI trend in Fig. 4A and Fig. 4B, with the exception of Fe-H which was significantly higher than Fe-L and Fe-M in the metal phase trial (Fig. 5A).

All metal treatments except U significantly reduced the number of mined petioles ($F(12,104) = 4.259$, $p<0.001$), and the Cu treatment had significantly fewer petioles mined than all the other treatments, except Zn and As (Fig. 6A). The control treatment on the other hand sustained significantly the highest mined petiole in this trial with the exception of the U treatment. A similar pattern emerged in the number of leaf scars counted, which were significantly fewer in the Cu treatment than all others except As, Mn-L and Mn-H ($F(12,104) = 2.1349$, $p<0.021$) (Fig. 6B) . However, only As and Cu had fewer feeding scars than the control treatment. Unlike the adult feeding scars, the larval mining was significantly different from that of the control, indicating that the larvae were more sensitive to the Hg than the adult weevil. Both larval and adult feeding in the iron and manganese dose response treatments did not show any significant difference between them (Fig. 6A and B).

Different spectral indicators of plant stress from the spectral measurements at the end of the experiment (week-9) were correlated to SPAD-502 readings, number of larval mined petioles and adult feeding scars and all relationships were positive and significant (Table 3). Indices based on the red edge bands (mNDVI, REP-LE, RE-NDVI and REP-Max FD) showed stronger correlation compared to the green band index (PRI). The spectral indicator, mNDVI showed the strongest correlation with all the variables, except the correlation of REP-Max FD with larval feeding which was greater than that with mNDVI. Of all the spectral indicators the PRI showed the weakest correlation (Table 3).

4 Discussion

The hand held spectrometer was able to distinguish plant stress caused by different metals of which Cu was the most stressful; and biotic stress caused by biocontrol was also visible.

5.1 Heavy metal-induced plant stress

Heavy metals, with the exception of micronutrients, are not known for any particular role in plant metabolic functions and among these are Hg, Au, U, and As (Dunn, 2007). Hence plant uptake of such elements occurs either by physiochemical adsorption on the roots surfaces or by passive transportation and retention in the roots of many aquatic plants (Dunn, 2007). The localization of the heavy metals in the roots of most aquatic plants enables them to avoid metal phytotoxcity effects. However, some heavy metals in the roots could indirectly stress plants by interfering with the passage and root uptake of important nutrient elements from the water. For instance the uptake of As includes an active apoplastic mechanism using the phosphate uptake path ways, through which some arsenic could also reach the aerial plant parts (Rahman and Hasegawa, 2011). In contrast the micronutrients Fe, Mn, Cu, and Zn have an essential metabolic role in plants through their involvement in the chloroplast or chlorophyll synthesis (Fe and Mn) or Enzyme, coenzyme, and hormone synthesis (Cu and Zn) (Dunn, 2007). Hence, their uptake by plants occurs mainly by active transport processes.

Nevertheless, Vesk *et al*. (1999), found that unlike, Cu, and Zn, the uptake of Fe in water hyacinth was more localized at the surfaces of the water hyacinth roots than towards the root centre. Iron-plaques formed by iron hydroxides as results of oxidation of the root zone by the plant or microbial metabolism contributes to the iron localization on the surface of the root (Vesk *et al*., 1999).

In the first three weeks plants of water hyacinth were generally tolerant to most heavy metals under which they were grown. However, different spectral stress indicators calculated in this study revealed that water hyacinth plants were consistently under severe stress of Cu, Hg, and Zn treatments (Figures 3A, 4A and 5A). Several studies have already established the appearance of blue shifts in the red edge as an indicator of plant stress associated either with deficiency or excess of organic and inorganic elements due to their association with plant chlorophyll content (Ayala-Silva *et al*., 2005; and Kooistra *et al*., 2004; Horler *et al*. 1980, 1983). A greater first derivative peak at ~ 702 nm (first peak) by the treatments Cu, Hg and Zn compared to the control treatment and their relative shift towards the shorter wave length opposite to the direction of the control treatment indicated decrease in canopy chlorophyll concentration and as a result plant stress from the heavy metals (Fig. 3A). The shift towards the shorter wavelength (blue shift) of the REP in the Cu, Hg, and Zn treatments was by about 5.5 nm and this could be implicated to the concentration of the these heavy metals in water hyacinth plant (Fig. 4C). Rock *et al*. (1988) found a blue shift of 5 nm in spruce and fir species as a result of airborne acid deposition plant stress. Similarly Ren *et al*. (2008) in a single element trial, using the REP and the blue shift were able to identify the relative concentration of lead (Pb) in the canopy leaves of rice during the early tillering stage. In another study Jago and Curran (1996) also showed that the peaks of 693 nm and 709 nm from stressed grass canopy spectral measurement growing on oil-contaminated sites. The adjusted normalized difference index ($mNDVI_{705}$) also reflected the pattern of the plant stress shown by the first derivative reflectance curve (Fig. 3A) and the REP calculated using the linear extrapolation (Fig. 4C), where treatments of Cu, Hg and Zn were indicated as the most stressful heavy metals to the water hyacinth plants. The three band indices mNDVI which consists two of the red edge and one of the blue bands has an advantage over the ordinary red edge spectral indicator (e.g. RE-NDVI) since it corrects leaf structural variation and other additives such as the back ground effects and produces a stronger correlation with canopy chlorophyll concentration (Tiana *et al*., 2011).

The canopy water stress of water hyacinth matches the results of the spectral indicators associated with leaf chlorophyll concentrations ($mNDVI_{705}$, REP-Max FD and REP-LE) where water hyacinth grown in the same metal elements (Cu, Hg, and Zn) had the lowest water band index which showed the water stress level. Claudio *et al*., (2006) used the WBI to estimate the evapotranspiration and the canopy water status of vegetations in semi-arid shrubland ecosystem in Southern California and found a strong link between canopy water content and the green canopy structure.

The spectral indicators for the plant stress consistently showed that among the different heavy metals tested, water hyacinth was only very sensitive to Cu, Hg and Zn, during the first three week of the metal phase trial.

5.2 Interaction of the biocontrol agents with heavy metals in plant tissues

Most spectral indicators that detect plant stress are associated with plant chlorophyll. Excess and deficiency of plant nutrients affect the plant chlorophyll. For instance deficiency of both nitrogen and magnesium results in the entire plant chlorisis since they are an essential component of chlorophyll, while the deficiency of Ca, K and P only results in a partial chlorisis Ayala-Silva *et al*., 2005. Spectral indicators of plant stress as a result of nutrient deficiency, therefore, follow the same spectral trends, where the REP shifts towards the shorter wavelength and this makes it difficult to distinguish between them (Ayala-Silva *et al*., 2005). The same applies with high concentration of heavy metal plant uptake, that reduces leaf chlorophyll by resulting in higher concentration of destructive oxyradicals compared to the antioxidant plant systems and cause “oxidative stress” that eventually impair photosynthesis (Smolders and Roelofs, 1996). Similarly pathogenic or insect damage on plants alters the physiological and chemical status of plants by changing the concentration of chlorophyll pigments, chemical concentrations, cell structure and nutrient and water uptake that affect the colour and temperature of the plant canopy (Raikes and Burpee, 1998). Such characteristic changes in the plant canopy as a result of biotic damage also produce spectral features similar to those of excess heavy metal plant uptake or plant nutrient deficiency.

The intensity of the plant stress after the addition of the weevils (week-9) deteriorated and there were more treatments in week-9 showing stress of water hyacinth plant compared to week-3 which was consistently limited to Cu, Hg and Zn treatments as the only plant stressors (Fig. 4B and D). The fact that the REP of the control treatment had contracted by ~ 8 nm in week-9, and the fact that the number of treatments on which the water hyacinth showed stress had extended to seven (including As, Fe-M, Mn-L and Mn-H) instead of only three in week-3, indicates that both larval and adult plant feeding had increased the intensity of the plant stresses (Fig. 3B and 4B and D). The feeding damage of the weevils in week-9 decreased leaf chlorophyll pigments and changed the canopy structure that resulted in increased reflectance in the visible range of light spectrum and decreased in the near infrared range respectively. Mirik *et al*. (2006a) using a hand held spectrometer also found similar reflectance pattern in the greenbug-damaged wheat canopy compared to the undamaged wheat canopies. The distinct appearance of the first derivative curve with an increase in the first peak and decrease of the second peak are linked to the reduction of chlorophyll content and cellular structure as a result of feeding stress by the weevils. Mirik *et al*. (2007) found that the aphid infested wheat had lower reflectance than the non infested wheat around the same wavelength of the second peak in the red edge of this study. The Cu treatment sustained the greatest blue shift increase of about 14.5 nm followed by As and Mn-H among others (Fig. 3B and D). However, the increased plant stresses of Cu and the stress caused by As treatment in week-9 were not consequences of the weevils' damage, since the adult and larval plant damages of the weevils in these two metal treatments were among the lowest (Fig. 6A and B). Hence, it suggests that even though to a lesser extent the weevil damage might have aggravated the severity of the plant stress, but primarily it occurred because of the prevailing metal-induced stress of Cu and As metals, which could have been translocated into the leaves in the latter stage after the third week of the experiment.

It is known that the plant uptake of phosphates is negatively correlated with that of As uptake (Mkandawire *et al*., 2004; Rahman *et al*. 2007). The fact that the As treatment was not significantly different from that of the control treatment in week-3, suggests that phosphates from the Hoagland solution used at the beginning of the experiment could have inhibited the uptake of As by water hyacinth until the complete removal of the phosphates from the water in the first three weeks. Wang *et al*. (2002) found that the uptake of the arsenate by As hyperaccumulator plant, *Pteris vittata,* dropped in the presence of phosphate and increased by 2.5 folds after the depletion of the phosphates in eight days. The distinctive spectral signature of the plants in the Cu treatment throughout this experiment is a clear indication of the amount of Cu concentration and the degree of its phytotoxicity in water hyacinth. de Almeida *et al*. (2007) showed that extended exposure of plants to Cu led to a plant growth and development disorder, with severe chlorotic symptoms, by inhibiting cellular elongation and interfering with a number of enzymatic activities that reduces the photosynthetic processes.

Similarly Maksymiec *et al*., (1994) found that increased levels of Cu reaching the plant's leaves resulted in photoinhibitory damage to photosystem-tow (PSII). Therefore, the severity of leaf chlorises in the Cu treatment compared to Zn and Hg treatments could possibly be associated with relatively higher Cu translocation into the leaves. Soltan and Rashed (2003) found more Cu concentration (43±7 µg of Cu g^{-1} dry matter) than Zn (11±14 µg of Zn g^{-1} dry matter) in the aerial parts of water hyacinth.

Generally the water status of the water hyacinth plants had significantly dropped in the second phase of the weevil trial, indicating the deterioration of the plant heath as a result of additional plant stress exerted by larval and adult weevil damage of the leaf. Nevertheless, the water band index of Cu, and Hg treatments in week-9 (the weevil phase) was not significantly different from that of the control treatment, unlike in the first three weeks of the metal phase trial (Fig. 5). This could be associated to the fact that there was more larval and adult feeding damage in the control treatment by the water hyacinth weevil compared to Cu treatment and Hg (at least for the larval feeding) (Fig. 6A and B). Plant damage by insect herbivory reduces the canopy water content of plants through increased transpiration Aldea *et al*. (2005). For instance Aldea *et al*. (2005) found a 45% water lose in the plants damaged by the herbivory of *Popillia japonica* (Japanese beetles) and *Helicoverpa zea* (Boddie) on a soybean plants compared to the control plant (no herbivory). Similarly Tiana *et al*. (2011) evaluated successfully the species-specific drought response by estimating the rate of transpiration and canopy water content in three different plant species, chamise (*Adenostoma fasciculatum*), redshank (A. *sparsifolium*), and manzanita (*Arctostaphylos pungens*) using the WBI.

The correlation of the spectral indicators of plant stress to the SPAD-502 reading and both larval and adult feeding showed that all indices could easily detect the biotic and abiotic stress of water hyacinth at a canopy level. Nevertheless, the red-edge normalized difference indices and the spectral indicators for the evaluation of the REP produced relatively stronger correlation compared to the other indices, of which $mNDVI_{705}$ was the strongest of all. Tiana *et al*. (2011) also found that the $mNDVI_{705}$ was correlated more strongly with chlorophyll than the RE-NDVI (R^2 of 0.83 and 0.73 respectively). This is due to the fact that addition of the third blue band (reflectance at the wavelength of 445 nm) in the $mNDVI_{705}$ which helps to eliminate the effect of surface reflectance and light scattering at the wavelength of 800 nm (Sims and Gamon, 2002). The correlation of the spectral indicators with both larval and adult weevil damage however, was not as strong as those with the SPAD-502 reading. Mirik *et al*.

(2006b) found that spectral indicators were strongly correlated with the greenbugs damaged wheat crops with correlation coefficient ranging from 0.82 to 0.98. The fact that the water hyacinth weevils in this study were feeding on heavy metal contaminated plants had generally reduced the intensity of the weevils' performance, which could possibly suggest why the correlation of the spectral indicators with the weevils' damage was not strong.

Plants that grow under heavily polluted conditions and particularly those which are accumulators or hyperaccumulators are proposed to be resistant to some natural enemies (Boyd, 2010). The toxicity and the deterrent value of different heavy metal contaminants to insect herbivores is variable and acts either by reducing feeding, retarding larval development or in extreme cases by intoxicating the insects, causing death (Davis *et al*., 2001). This study suggests that the weevil larvae are more sensitive to Zn metal accumulation in the plant tissue than the adults of the weevils, whereas Cu and As metals were both deterrent to the adult and larval feeding (Fig. 5A and B). Morgan and Trumple (2010) in their review study indicated that the feeding damage of *Neochetina bruchi* had significantly decreased compared to the control treatment when fed to plants with 232 μg Zn/100g dry weight. On the other hand Kay and Haller, (1986) found that the damage incurred by *N. eichhornia* growing on a concentration of 2.5 mg/L of water was not significantly different from those of the control treatments. This however, contradicts our results which showed that both larval and adult feeding of the weevils (both *N. Eichhornia* and *N. bruchi*) had incurred significantly lower feeding damage on the plants compared to theirs despite the fact that water concentration used to grow the water hyacinth in this study was only 2 mg/L (less than that used by Kay and Haller, (1986)). However, this could be associated to the fact that our experiment was run for a longer period (six weeks) as opposed to that of Kay and Haller, (1986) which was only for one week.

6 Conclusion

The hyperspectral data was convincingly able to distinguish the intensity of the stress caused to water hyacinth plants under different biotic and abiotic treatments and this indicates that it has potential as an effective tool that could be used to determine the health status of water hyacinth from a remote position. The water hyacinth plant was generally tolerant to the heavy metals with the exception of Cu, Hg and Zn treatments, which consistently revealed stressful spectral features when analysed using different spectral stress indicators. The spectral indicators of plant stress also showed that the same metals reduced the weevil's performance of which Cu was the most stressful to the weevil. It is therefore, advisable to implement water hyacinth control method other than biocontrol agents for effective and successful results, in water systems contaminated with heavy metals and particularly those waters contaminated with copper. This study was conducted at small scale in tubs and therefore, investigating the interaction of the heavy metals with the water hyacinth weevils in field environment is recommended.

Table 1: Heavy metal treatments and their concentrations used in the tub experiment.

Salt compound	Molecular weight	Conc. of stock solution in g l^{-1}	Metal concentration in the stock solution prepared		Volume of the stock solution added per tub (ml)	Final metal concentration in tubs (mg l^{-1})
			Elements	mg l^{-1}		
As_2O_3	197.84	1	As	757.4	55.45	1
$AuCl_3$	303.33	0.2	Au	129.87	32	1
$Cu(NO_3)_2.3H_2O$	241.6	1	Cu	263	319.4	2
$Fe(NO3)_2.H_2O$	404	0.5	Fe	69.11	303.86	0.5
$Fe(NO3)_2.H_2O$	404	2	Fe	276.46	303.84	2
$Fe(NO3)_2.H_2O$	404	4	Fe	553	303.8	4
$Hg (NO_3)_2.H_2O$	342.62	0.5	Hg	297.7	143.5	1
$MnSO_4.H_2O$ (1)	169.02	1	Mn	325	64.6	0.5
$MnSO_4.H_2O$ (2)	169.02	1	Mn	325	258.5	2
$MnSO_4.H_2O$ (3)	169.02	2	Mn	650	258.5	4
Uranium						1
$N_2O_6Zn.6H_2O$	297.48	3	Zn	659	254.93	4

NB: the suffix numbers (1), (2) and (3) in the first column refers to Low, Medium and High concentrations respectively

Table. 2: The spectral indices used to analyse heavy metal and the water hyacinth weevil induced plant stresses.

Indices	Name	Formula	Reference
RE_NDVI	Red edge Normalized difference vegetation index	(P750-P705)/(P750+P705)	Gitelson and Merzlyk, 1994
$mNDVI_{705}$	modified red edge normalized difference vegetation index	$(P_{750}-P_{705})/(P_{750}+P705-2P_{445})$	Datt, 1999
PRI	photochemical Reflectance Index	(P531-P570)/(P531+570)	Gamon *et al.*, 1992
mSR	Modified Red Edge Simple Ratio Index	(P750-P445)/(P705-P445)	Sims and Gamon, 2002
REP_MAX-FDR	Red edge position: maximum first derivative wavelength	$FDR_{(\lambda i)} = (R_{\lambda(j+1)} - R_{\lambda(j)})/\Delta\lambda$	Dawson and Curran, 1998
REP_LE	Red edge position: linear extrapolation method		Cho and Skidmore, 2006
WBI	Water Band Index	P900/970	Peñuelas *et al.*, 1995b

Table. 3: Correlations of larval mined petioles, adult feeding scars and leaf chlorophyll measured with a SPAD-502 and spectral plant stress indicators of water hyacinth grown in tubs with heavy metal and weevil treatments in week-9. $P<0.001$.

Spectral Indices (R^2)	SPAD-502 (R^2)	Larval feeding (R^2)	Adult feeding (R^2)
PRI	0.608	0.1481	0.1523
mSR	0.675	0.1574	0.3579
REP-Max FD	0.703	0.27	0.3348
RE-NDVI	0.749	0.1804	0.3552
REP-LE	0.754	0.1816	0.3557
mNDVI	0.791	0.2006	0.3694

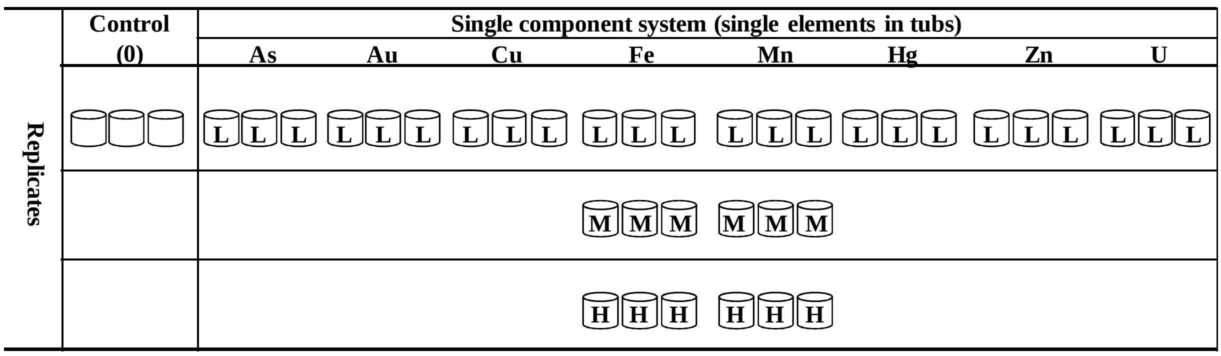

Fig. 1: Experimental design of the single-component system tub trial centrations of heavy metals. NB: L = low, M = medium and H = high concentrations.

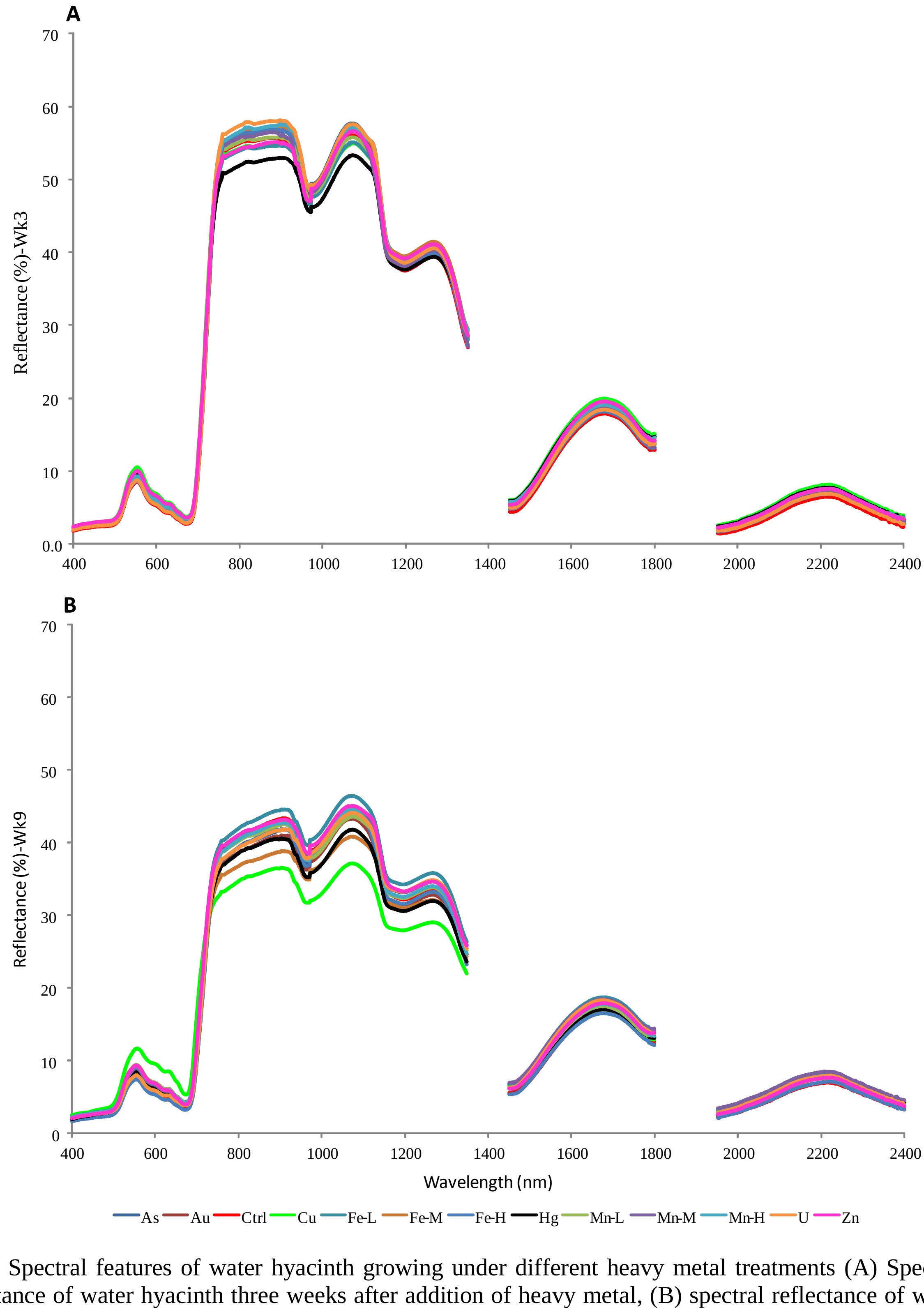

Fig.2: Spectral features of water hyacinth growing under different heavy metal treatments (A) Spectral reflectance of water hyacinth three weeks after addition of heavy metal, (B) spectral reflectance of water hyacinth six weeks after the addition of weevils

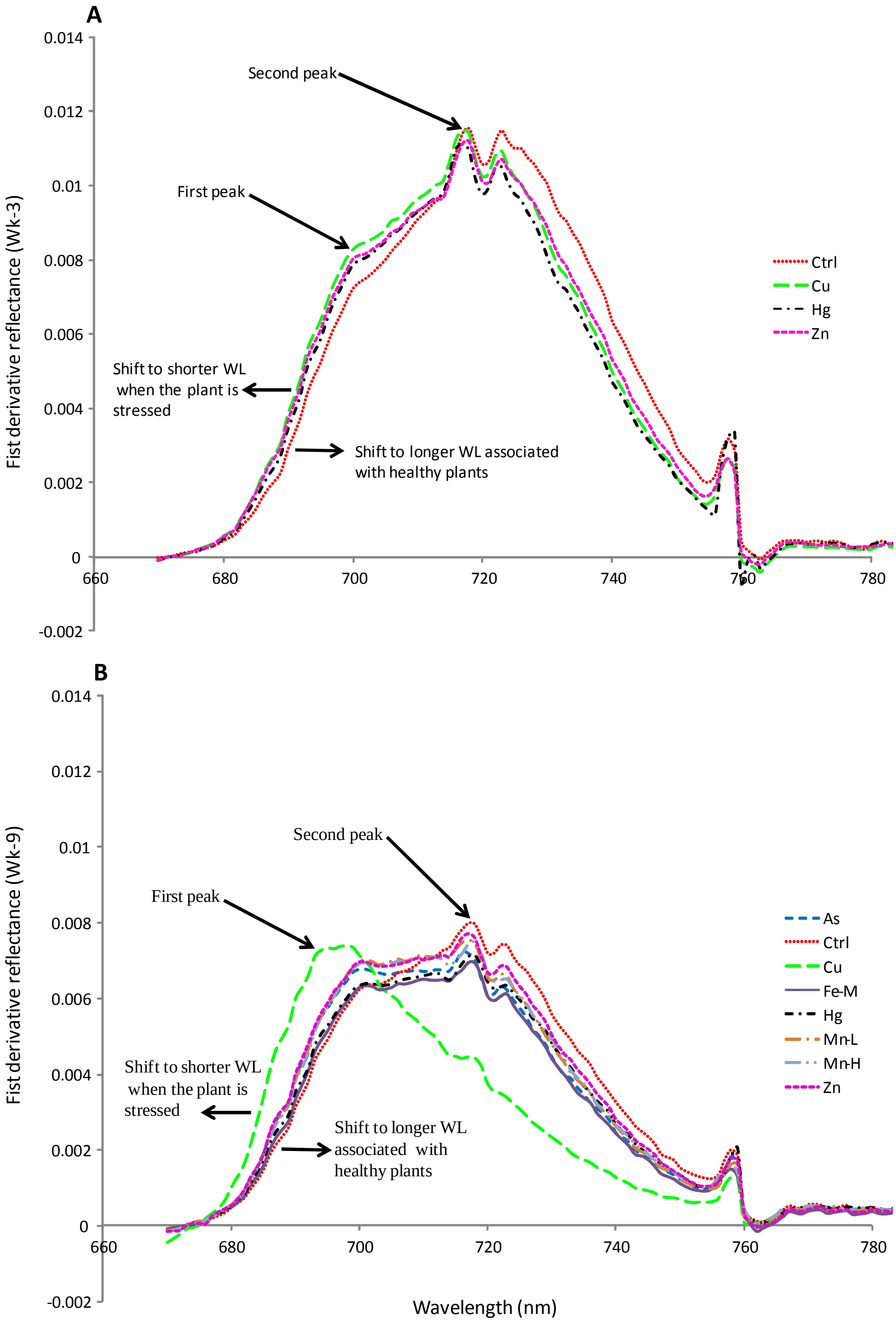

Fig.3: Spectral features of water hyacinth growing under different heavy metal treatments (A) First derivative curve for Wk-3 (B) first derivative curve for Wk-9, which is six weeks after the addition of weevils.

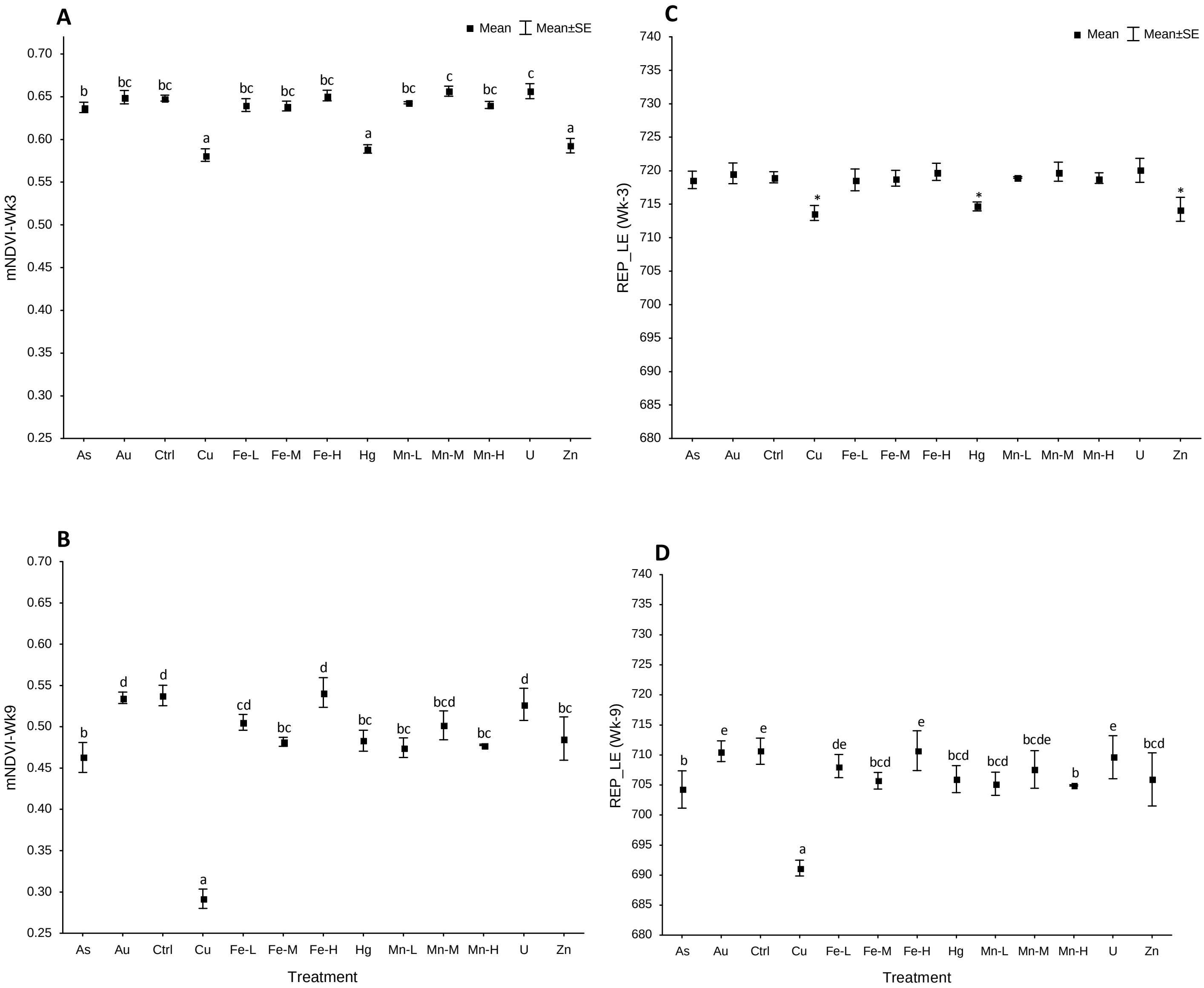

Fig. 4: Evaluation of plant health status of water hyacinth grown under heavy metal and weevil stressors using the spectral stress indicator: (**A**) $mNDVI_{705}$ to detect heavy metal-induced plant stress in week-3 (**B**) $mNDVI_{705}$ to detect weevil-induced plant stress in week-9 (**C**) REP_LE to detect heavy metal-induced plant stress in week-3 and (**D**) REP_LE to detect weevil-induced plant stress in week-9. Means were compared by One-way ANOVA and those followed by the same letter(s) are not significantly different (P>0.05; Fisher LSD test).

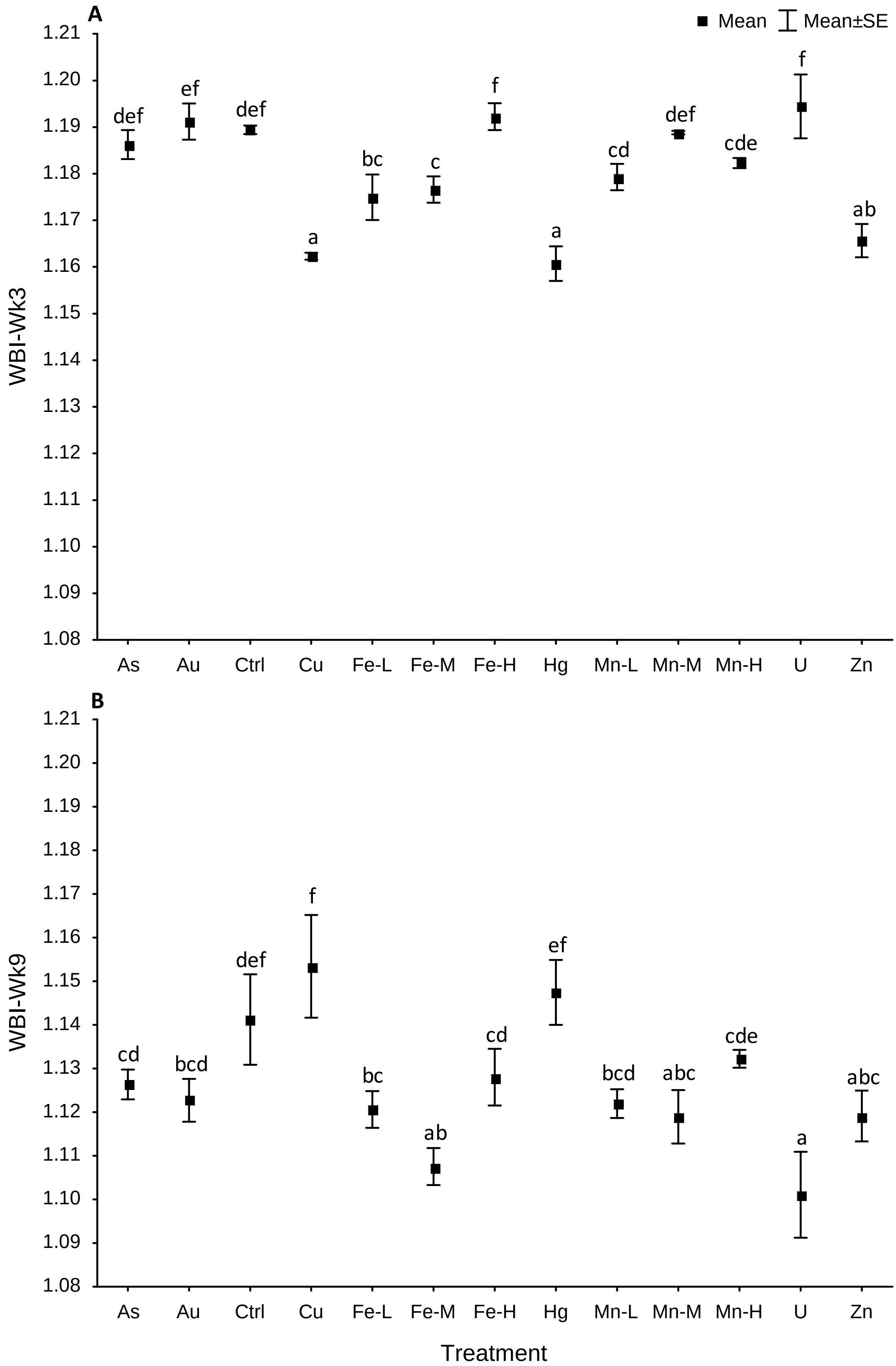

Fig. 5: Evaluation of canopy water content of water hyacinth grown under heavy metal and weevil stressors using the water sensitive spectral index, WBI (**A**) Heavy metal-induced plant stress in week-3 and (**B**) water hyacinth weevil-induced plant stress in week-9. Means were compared by One-way ANOVA and those followed by the same letter(s) are not significantly different (P>0.05; Fisher LSD test).

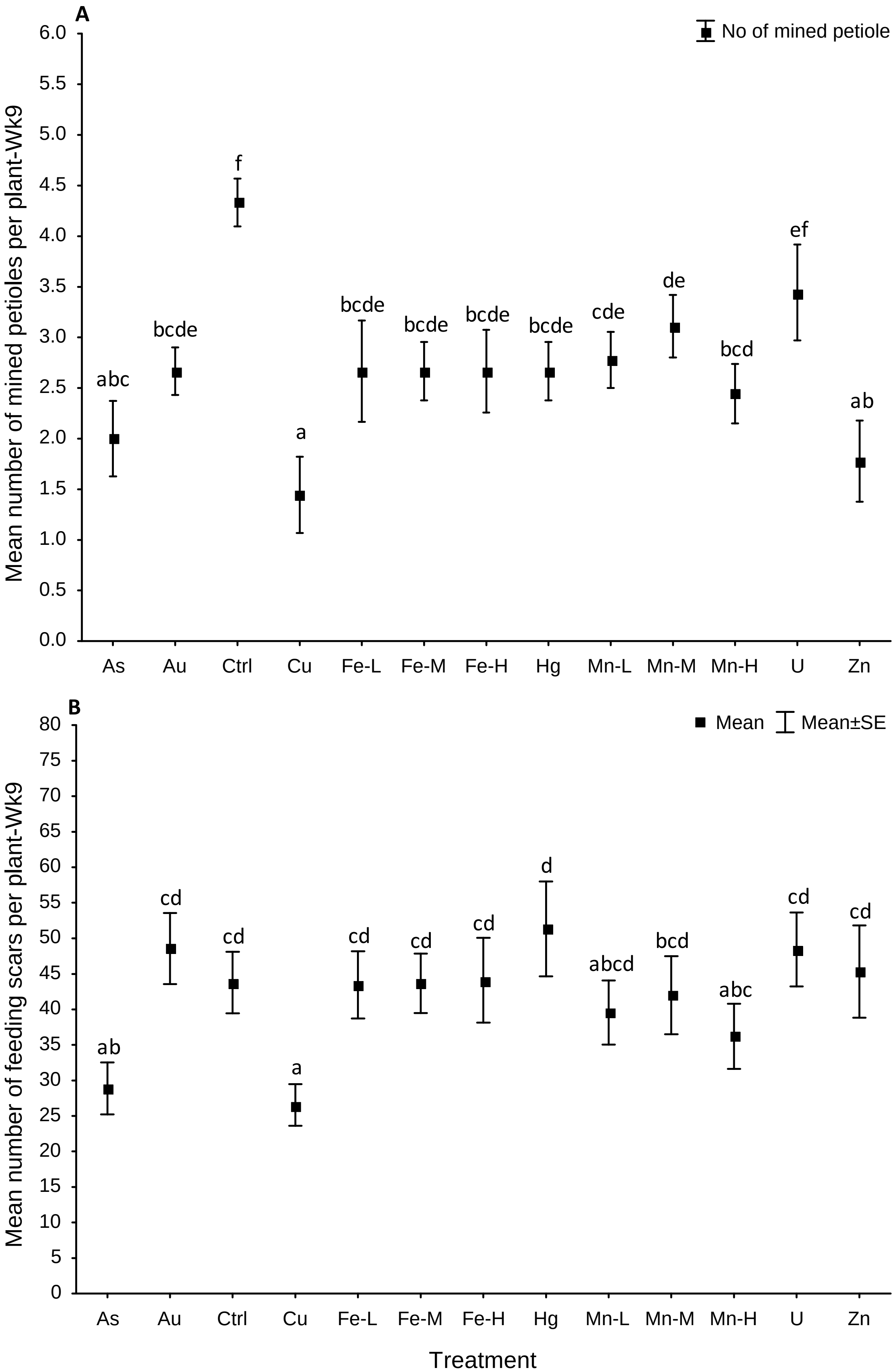

Fig. 6: The interaction of a biocontrol agent with heavy metals on water hyacinth growing under different heavy metal treatments in tubs: (A) Mean number of larval petiole mining per plant-Wk9 and (B) Mean number of adult feeding scars per plant-Wk9.