



BIOLOGICAL ASSESSMENT OF THE EFFECTS OF TOXIC METALS ON PLANT BIOMASS PRODUCTION

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Abstract

A significant environmental problem is the disturbance of acid sulfate soils which contain high amounts of sulfuric acid or have the potential to form it, resulting in pollution of the environment due to mobilization of toxic metals into soil and water systems. Reported in this study are two bioassay experiments carried out using wheat plants (*Triticum* sp.) to investigate the potential causes of a significant environmental problem experienced in a farmland. The results obtained show that availability of excessive amounts of Fe in the rhizosphere and formation of Fe-complex plagues, either acted as barriers to nutrient uptake or enhanced uptake of potentially toxic metals in excessive amounts; coupled with the presence of toxic levels of Al killed the vegetation on the farmland.

Keywords: Bioassay, toxic metals, environmental problem.

Introduction

A significant environmental issue is the oxidation of acid sulfate soils (ASS) containing high levels of iron sulfide minerals, predominantly pyrite (FeS_2) (Dent and Pons, 1995; Fitzpatrick et al., 2008; Pons, 1973; Wilson, 2005) which has the potentials to cause soil and water acidification when oxidised (Appleyard et al., 2004b; Macdonald et al., 2004). The exposure of the ASS by drying or falling water levels have profound effects on ground and surface water, their quality and utilization (Appleyard et al., 2004b; Fitzpatrick et al., 2009; Haling et al., 2011). When the ASS are exposed, aeration of acid sediments and oxidation of FeS_2 occurs. Upon rehydration, sulfuric acid is formed, which when released can mobilize naturally occurring toxic metals and transport them, leading to environmental (soil and water) pollutions (Fitzpatrick et al., 2010; Michael, 2013; Michael et al., 2012).

Pollution of soil, water and surface vegetation by toxic metals released is a serious problem (Appleyard et al., 2004a; Hinwood et al., 2006), and attempts are made to assess the level of pollution or to identify the cause of it (Havel, 1997). Nowadays, the method used to identify the cause (pollutants) of a specific environmental problem requires sophisticated and

specialized equipment. However, biological assessment (bioassay) - the observation of pollutants' effects on an organism requires little equipment and can be adapted to many conditions (Havel, 1997). Testing the effects of pollutants on an organism such as a plant species has increased in popularity because of the invaluable information that can be gained regarding the interdependence of living organisms, their environments and the effects of pollutants on them (Havel, 1997).

Recently, Environmental Protection Authority (EPA), South Australia has reported that a farmland in the Lower Murray River Basin, South Australia (Figure1) was polluted by an unknown soil-water condition, which resulted in the farm being abandoned. In addition, preliminary data from water sample collected from one of the drains in the farm and analysed showed the presence of possible pollutants (Table 1), however; the actual causes were not quite understood, hence this study.

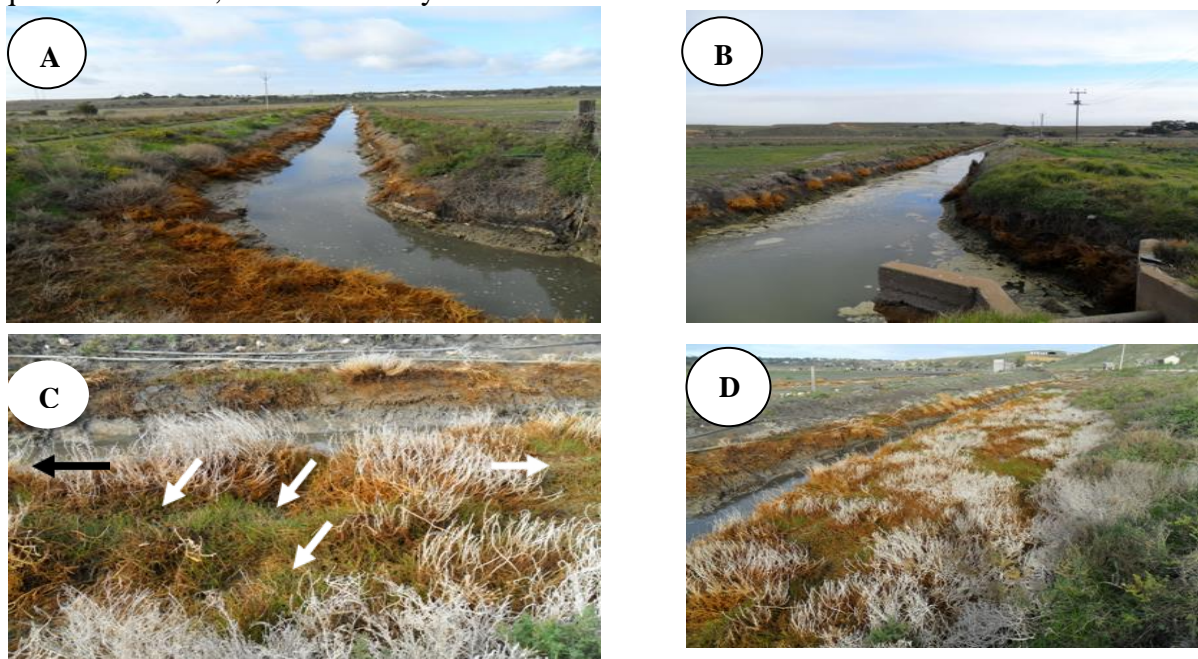


Figure 1: Photographs (A-D) show polluted drains with dead vegetation on the farmland. The arrows show the plant tissue (white) and water (black) sampling locations

Materials and Methods

Prior to the experiments, the farm was visited, field observations made, and water and plant tissue samples taken. Based on the observations made during the field visit, two lab-based experiments were carried out. In the first experiment (hereafter experiment I), the effects of polluted water samples from Toora on the growth performance of wheat plants (*Triticum* sp.) were investigated, and in the second experiment (hereafter experiment II), the effects of four toxic metals (aluminium, iron, cobalt and manganese), found to be present in higher concentrations in the drain water of the same site, that were thought to be responsible for polluting the farm were tested; also using wheat plants.

Table 1: Contaminated water sample and plant tissue data

Key: < = below detection limit.

Location	Plant species	Sample size (g)	Metal concentrations (mgkg ⁻¹)			
			Fe	Al	Co	Mn
Upper	<i>Triglochin</i>	0.407	440	470	<0.7	55
Lower	<i>Triglochin</i>	0.405	7700	580	10	138
Upper	<i>Samphire</i>	0.412	142	50	<0.7	25
Mid	<i>Samphire</i>	0.416	94	36	<0.7	57
Mid	<i>Samphire</i>	0.409	14700	9000	112	4000

Water and Plant Tissue Sampling

Preliminary determination of the drain's water and soil pH were done to confirm the presence of acid in the water or soil. Contaminated water samples together with tissues of *Triglochin* sp. and samphire (*Salicornia europaea*) plants, growing along the contaminated drain, among and few centimetres away from the death plants were sampled (Figure 1). The water samples taken were used in experiment I while the plant tissues were used for qualitative analysis of the presence of metals in the tissues (Table 1), for the data to be used in setting up experiment II. For both of the plants, tissues were taken from a lower and a middle location, except for samphire, where a sample from an upper location was also taken, and included (Figure 2).

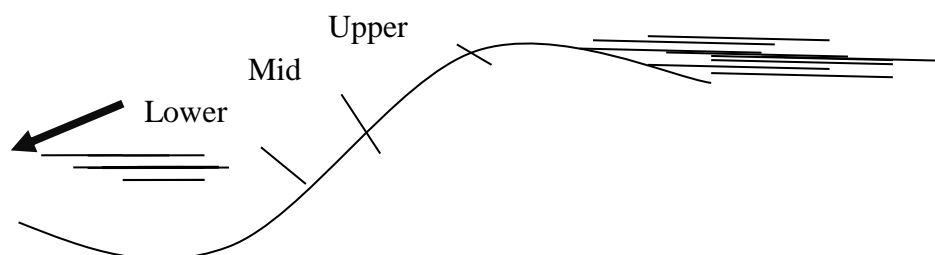


Figure 2: The locations of plant tissue sampling. The arrow depicts the direction of the drain and the water sampling site. Note: Location not drawn to scale

Two 20L containers were used and water samples collected from the nearby drain. Both of the containers were labelled with name of the sampler, the site and the sampling date; and brought to the lab for use. Water samples were collected from the same site previously by the EPA to analyse the presence of toxic metals (Table 1). The sampled plant tissues were kept in sampling bags, labelled in a similar manner as the water samples, and also brought to the lab for analysis by the EPA using inductively coupled plasma atomic emission spectroscopy (ICP AES).

Seed Germination and Seedling Preparations

To quantitatively confirm the pollutants present in the polluted water; wheat seeds were pre-germinated over three nights under lab conditions to be used. To do this, two separate seed

germination and seedling preparation exercises were carried out. In both experiments, a good number of seeds were placed on double-folded tissue papers, moistened with deionised water (DH₂O) while in a rectangular germination tray, and incubated under lab conditions to allow the seeds to germinate over three days.

In experiment I, the three-day old seedlings were isolated carefully from the germination tray and their roots dipped in a vial containing DH₂O to soften them up. The roots were then blot dried on tissue papers, straighten up and transferred into several Eppendorf tubes (a seedling per tube), using the aid of forceps; prior to transferring them to a 20% Hoagland Nutrient Solution (hereafter 20% HNS). In experiment II, the seedlings were pre-germinated in the same manner except that they were kept overnight in a solution containing 0.1% HNS, before transferring to the 20% HNS; due to unavailability of stock solutions. All the tubes containing the seedlings (in both experiments) were then partly suspended in a germination box containing the 20% HNS, making sure the roots were directly in contact with the nutrient solution through several holes in the lid. The nutrient solutions were aerated and incubated for another three days under lab conditions to allow the seedlings to grow further; prior to transplanting them in the different treatment solutions.

Quantitative (Bioassay) Analysis

For experiment I, the six day-old seedlings were taken from the 20% HNS, their roots carefully washed with DH₂O and dried using tissue papers. These seedlings were then weighed with their tubes, one at a time; prior to transplanting them in the treatment solutions. All the seedlings were assigned numbers 1-5, and the data collected were recorded and tabulated for later use as per these numbers. For experiment II, treatment solutions were prepared separately; based on the water sample data provided the EPA.

The solutions of experiment I contained either a 35 ml of 20% HNS in 315 ml of DH₂O [three (15cm tall) vials containing 15 seedlings (Figure 3A)], 35 ml of 20% HNS in 315 ml of contaminated water [(CW) two vials containing 10 seedlings (Figure 3D, _{1, 2})] or a 350 ml of CW without any nutrient solution at all [one vial containing 5 seedlings (Figure 3D, ₃); the former being the control and the last two as treatment solutions, respectively. In experiment II, the treatment solutions were prepared as follows: the control solution contained 35ml of 20% HNS in 315 ml DH₂O; and for rest of the treatment solutions each contained a 20 mg of aluminium chloride (AlCl₃), 16 mg of iron sulfate (FeSO₄), 23.8 mg of cobalt chloride (CoCl₂) and 198 mg of manganese chloride (MnCl₂) in 35 ml of the 20% HNS in 315 ml of DH₂O.

For cobalt and manganese, stock solutions of 10 mM and 100 mM were prepared from which the required amount of 0.29 ml (0.29 mM) and 28.6 ml (28.6 mM) respectively were supplemented, in each of their respective treatment solutions. All the metal sources used are hereafter referred to as aluminium (Al), iron (Fe), manganese (Mn) and cobalt (Co). A small amount of ethylene diamine tetra acetic acid (EDTA) was however, supplemented in each of the treatment solutions including the control as Fe source. Each vial was planted with five seedlings of equal sizes, based on the numbers (1-5) assigned previously; in an anticlockwise direction, starting from the aeration holes in each vial. The vials containing the seedlings were labelled as per the treatments, and cultured for a week by keeping them in a culture room, with 14 hours of light (day length) and 8 hours of darkness at 24±2⁰C.

Harvesting and Data Collection

Observations on the plants were made at two days interval throughout the week until the plants were harvested. At harvest, the vials with the plants were taken to the lab, the plants removed from their vials and carefully separated into individual plants, especially between and among plants where the roots of a plant were entangled with those of other plants. The isolated plants were then blot dried using tissue papers and weighed whilst still in their tubes; one at a time by carefully placing a plant each on a weighing boat using a digital balance. The masses of the plants were then recorded against their assigned numbers. This was followed by weighing of the respective tubes of each plant until all the plants, and their tubes respectively were weighed, and recorded likewise.

Data Analysis

The data collected were tabulated and analysed using Microsoft Office Excel 2007. The growth of each plant, in terms of mass gains, was determined in the following manner: firstly, the mass of a tube was subtracted from the initial mass (the total mass of a seedling and its tube) to get the initial mass of a seedling that was in that tube, and secondly, the initial mass of the seedling was subtracted from the final mass of that plant recorded during harvest. The difference between the final and the initial mass of a plant was recorded as the growth mass of that plant (biomass production), measured in grams (g). The growths of all the other plants were also determined in this manner, until all the plants' final masses were taken and recorded against their assigned numbers.

Results

Field pH Measurements

The preliminary results of field-based pH measurements using soil samples in water (soil: water, 1:5, w/w) were near neutral or alkaline ($\text{pH} \geq 7$; data not shown). This indicated that the pollution effect on the vegetation along the drains was due to some other sources of pollutants present in the drain water or from the paddock.

Water Sample and Plant Tissue Data

The analysed CW data obtained from the EPA showed that there was a high concentration of metals, among others; Al (10.23 mgL^{-1}), Fe (93.70 mgL^{-1}), Co (0.79 mgL^{-1}), and Mn (7.20 mgL^{-1}). The ICP AES analysed plant sample data are given in Table 1.

Effects of CW on Plant Biomass Production – Experiment I

The observations made in experiment I showed that the plants grown in treatment solutions containing 350 ml of CW or 315 ml CW together with 35 ml of 20% HNS had totally retarded the growths of all the plants (Figure 3D, 1, 2, 3), compared to the control treatments (Figure 3C) within seven days. It was seen that leaf necrosis started from the tip of the older leaves in most of the plants and progressed down the lamina. Towards the end of the week, leaves of such plants were completely wilted, dry or both, and most of them were death

(Figure 3D). The roots of such plants were also thin, weak, short, and the tips were slightly swollen, greyish in colour; and were coated with iron oxide (Figure 3F); compared to those of the control treatment solutions, which were white and quite extensive (Figure 3E).

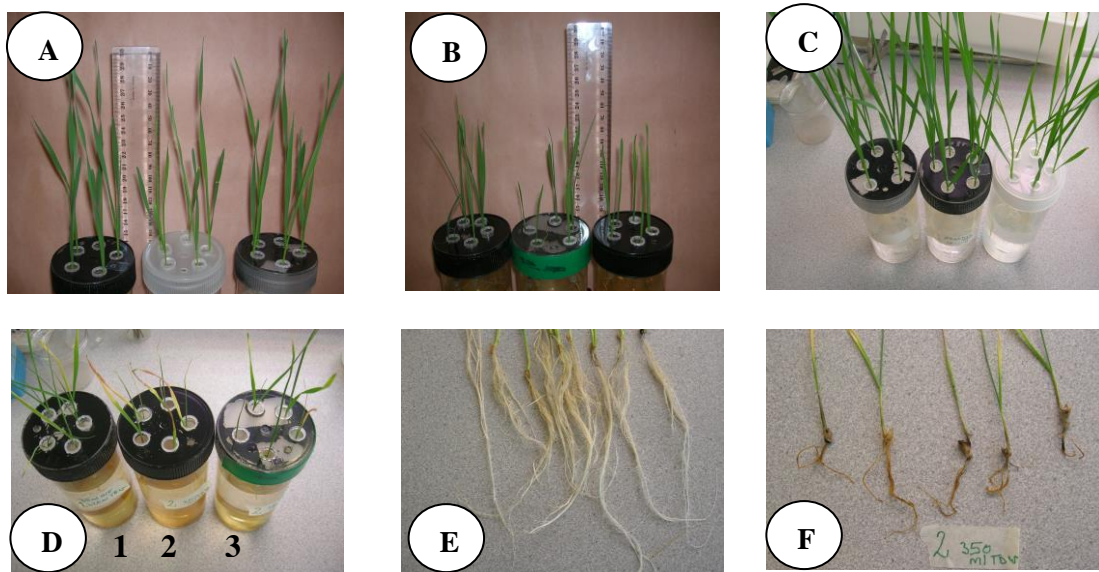


Figure 3: Plant biomass production in experiment I. A, plants growing in the control and B, in the treatment solutions three days after incubation; C, seven day-old plants in the control and D, in treatment solutions respectively. E and F show the root performances of the control (A) and treatment (B) plants. D_{1, 2, 3} as described previously.

The measurements made showed that the plants grown in control solutions using DH₂O as contaminant free containing 20% HNS resulted in production of large plant biomass (large leaves, stems and roots; Figure 3C), compared to the plants with access to the CW with the same amount of 20% HNS. The mean plant growth data of the control and treatment solutions are given in Figure 4. It was seen that the mean plant growth of the treatment solutions, containing either 350 ml of CW alone or in combination of 315 ml CW and 20% HNS were relatively smaller (less plant biomass production) compared to those of the plants in the control solutions, indicating that the contaminated water or a contaminant in the CW was responsible for the problem observed in the subject farmland (Figure 1).

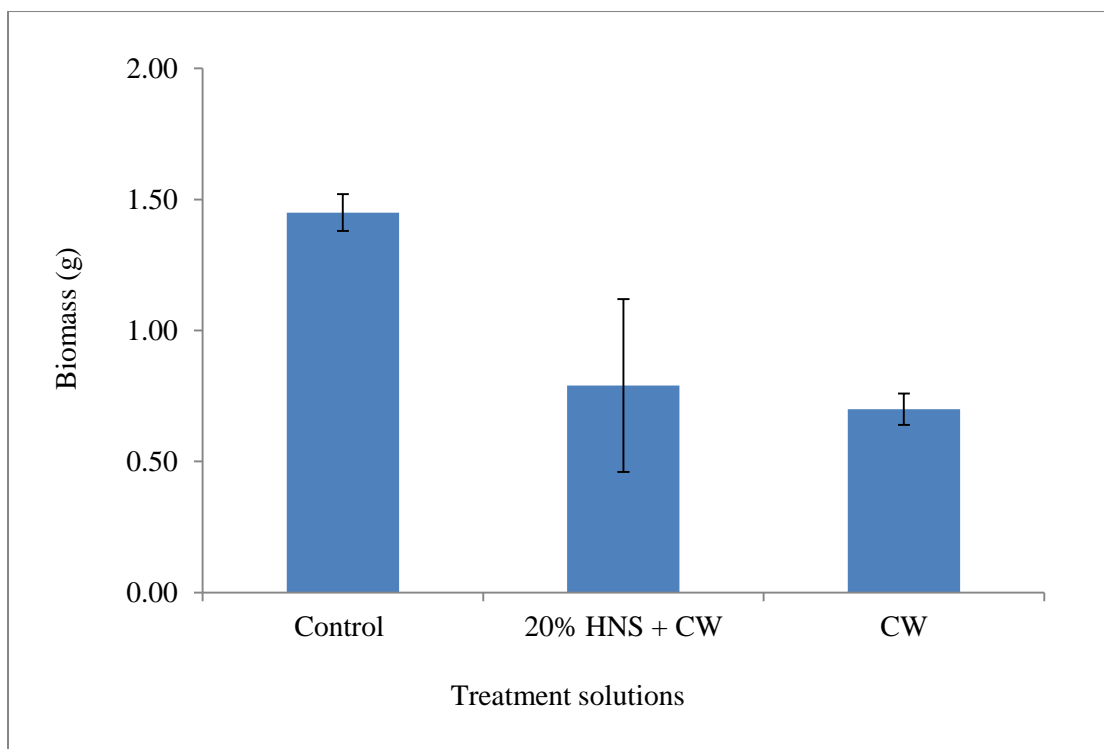


Figure 4: Plant biomass production in experiment I. Error bars are with standard error.

Effects of Metal Contaminants on Plant Biomass Production – Experiment II

In experiment II, it was observed that the plants grown in the treatment solutions containing Fe and Al had positive effects on the growth of the plants; hence plant biomass (Figure 5A, 4, 5). Most of the plants' growth parameters (leaf length, height, root lengths were retarded (data not shown). The leaves were necrotised from the lamina, down to the lower parts. Comparatively, the plants grown in the solution containing Co and Mn were similar to that of the plants in the control solution. Plant growth was also good with huge mass of leaves and roots (Figure 5A, 2, 3), similar to those of the control (Figure 5A, 1). The root lengths of these plants were also extensive and white (Figure 5B, 3).

On the other hand, the roots of plants grown in the solution containing Fe (Figure 5D) and Al (Figure 5C) were comparatively reduced and retarded. The roots of plants grown in the solution containing Fe were coated with iron-oxide (orange-red) and all such roots were death, dying or both; in both experiments (Figure 3F and Figure 5D). In the solution containing Al, plant roots were also reduced and somewhat retarded, except that they were fresh and healthy. In these plants, multiple roots have been induced (Figure 5C) compared to the plants in the other two solutions containing Co and Mn (Figure 5B, 1, 2). Comparative morphological analysis of the plant biomass produced in the solutions containing Co and Mn showed the plants were similar to those plants in the control solution (Figure 5A, 5B).

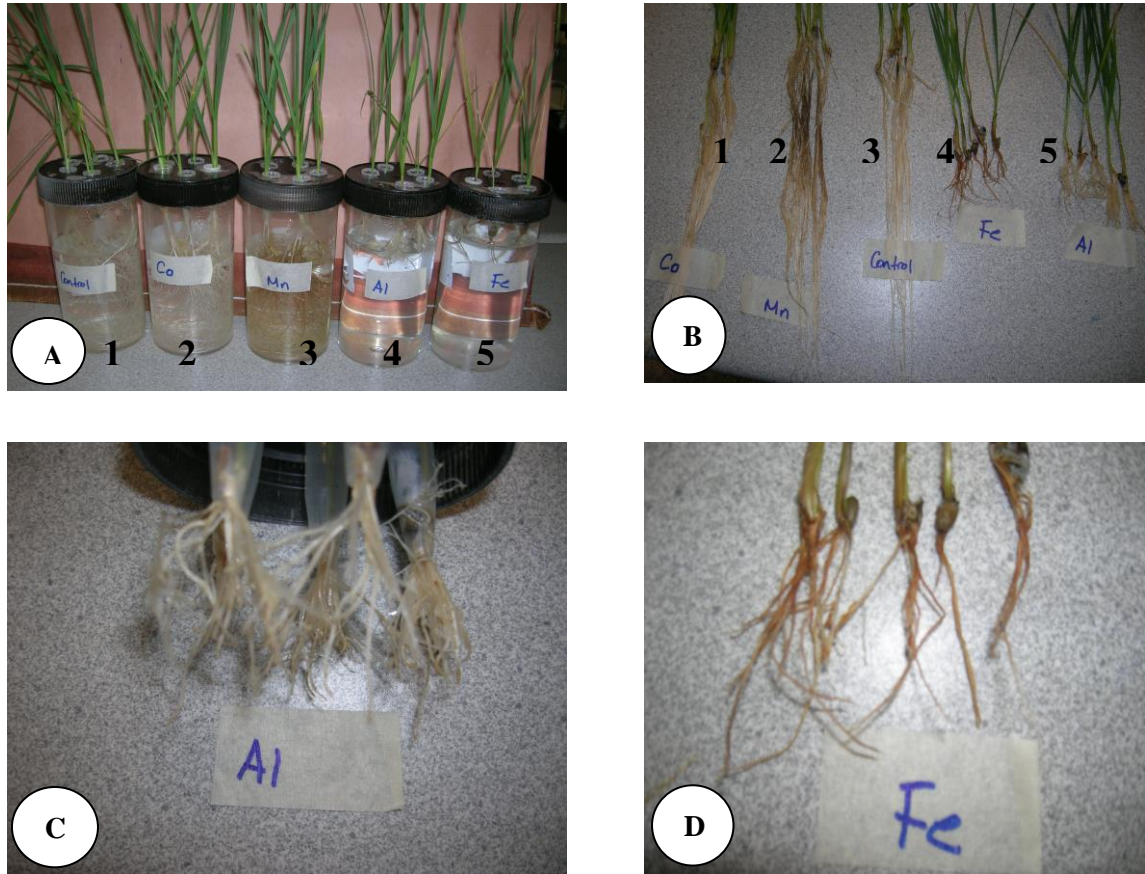


Figure 5: Plant biomass production in experiment II. A, the shoots; B, the roots; C, multiple roots induced in the presence of Al and D, roots coated with iron-oxide.

Plant Biomass Data

In experiment I, the mean growth masses of the plants in the control solution was 1.45 g, whereas for the plants grown in the solutions containing 35 ml 20% HNS and 350 ml CW were 0.79 g and 0.70 g respectively (Figure 4). Similarly, in experiment II, the mean growth masses of the plants grown in the control solution was 1.67 g, Mn was 1.72 g, Co was 1.70 g, Al was 1.14 g and Fe was 0.91 g (Figure 6).

The standard errors of the means (SEMs) for experiment I were as follows (Figure 4): the treatment solution containing 35 ml 20% HNS in 315 ml CW was 0.09 (with 95% confidence interval (mean \pm 1.96*SEMs) for the mean 0.79 \pm 0.18); the control solution was 0.07 (1.45 \pm 0.14) and finally the solution containing 350 ml CW were 0.02 (0.70 \pm 0.04). In experiment II, the SEMs were as follows (Fig.6): the solution containing Co was 0.27 (1.70 \pm 0.35), control was 0.16 (1.67 \pm 0.31), Mn was 0.11 (1.75 \pm 0.22), Al was 0.11 (1.14 \pm 0.22) and Fe was 0.07 (0.92 \pm 0.14).

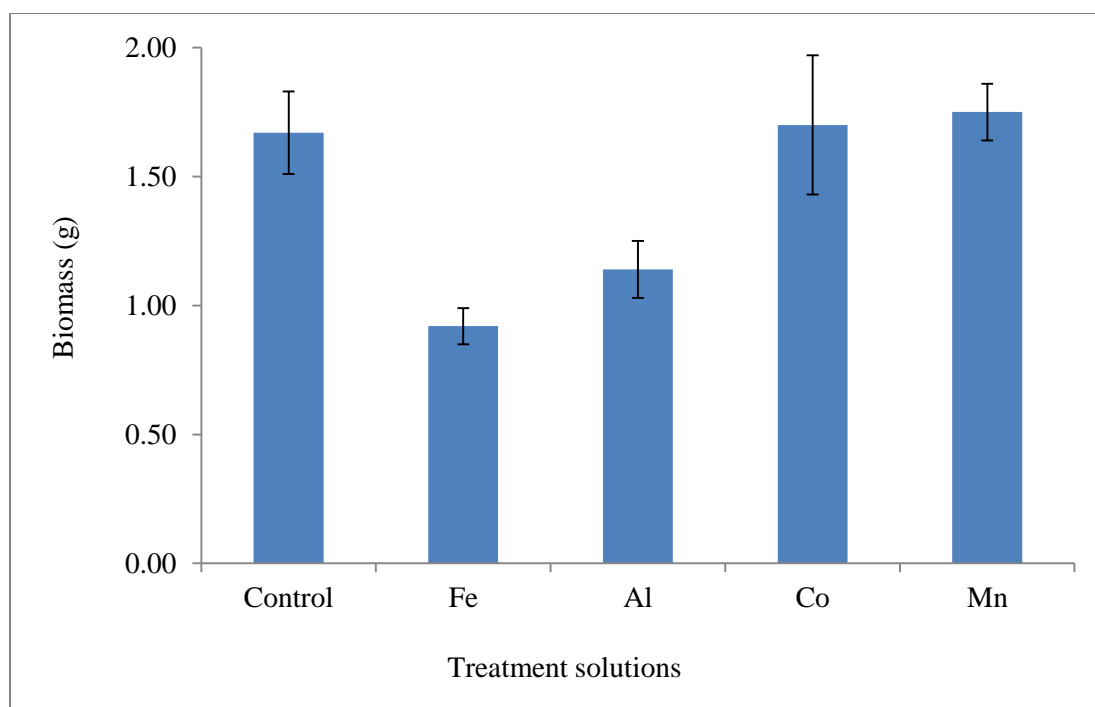


Figure 6: Plant biomass production in experiment II. Error bars are with standard error.

Discussion and Conclusion

The pH measurements using soil samples made in water in the field showed that neither the soil nor water was acidic. This has indicated that some other sources of toxicity, either in the soil or water were responsible for the death of the vegetation along the drainage systems of the farm. Obviously, the iron-oxide coatings on the dead vegetation along the drains have shown that there was excess Fe in the soil and drain water (Figure 1), which could have been the probable cause of the pollution. To that effect, the water sample data obtained from EPA has additionally shown that Fe, Al, Mn and Co concentrations were high, among other metals in the drain water. Moreover, analysed plant sample data from the site (Table 1) has also shown that Fe content in the plant tissues of both plants were higher, especially from the plants that were closer to the contaminated drain water than the ones that were further up the drain (Lower and Upper respectively, Figure 2).

The result of experiment I showed that the treatment solution containing CW alone (350 ml) had a positive effect on the growth of the plants (Figure 3D, ₃), compared to the treatment solution containing 35 ml of 20% HNS in 315 ml CW (Figure 3D, _{1, 2}) and the control solution containing 35 ml 20% HNS, and 315 ml DH₂O (Figure 3A, C) respectively. Also, experiment II (Figure 6) showed comparatively that the Fe treatment solution completely retarded the growth of all the plants (Figure 5A, ₅), followed by Al (Figure 5A, ₄), whereas that of the Co (Figure 5A, ₂) and Mn (Figure 5A, ₃) were similar to the control solution (Figure 5A, ₁). As a result, the growth performances of the plants in the latter two solutions were similar to that of the control.

As observed in the Al treatment solution with the exception of multiple root formation (Figure 5C), Harling and co-workers (Haling et al., 2011) reported that Al toxicity constrains root elongation and hence plant growth, thereby compromising nutrient and water uptake for

plant productivity (White and Kirkegaard, 2010). A recent study by (Zhengguo et al., 2009) who conducted a Mn waste rock pot experiment using capsicum plants reported that Mn was beneficial to increase chlorophyll content, stem height, mass and fruit, a result consistent with the finding in this study where Mn has had no effect on the plant growth and development. Similarly, the increase of Mn solubility by chemical mobilisation could promote Mn absorption, hence healthy plant growth (Sadana et al., 2005). Co was reported to inhibit roots, coleoptiles, and hypocotyls of germinating wheat seeds (Munzuroglu and Geckil, 2002) and a previous study indicated that Co is essential only for some plants, and it affords both beneficial effects and toxicity to plants (Öncel et al., 2000), as opposed to the results obtained in this study.

Observations made on the plants' growth parameters, especially the roots that were directly in contact with the treatment solutions of both the CW (experiment I) and the treatment solution containing Fe (experiment II) were coated with Fe-oxides, were weak and death or both (Figure 3F & Fig.5D, respectively). Maximum production of the coating (Fe plague) on roots occurs at the peak of plant biomass production (Crowder and Macfie, 1986) and mainly consists of Fe(III)-oxo-hydroxides (Taylor et al., 1984) as a result of microbial oxidation (Levan and Riha, 1986) and radial oxygen loss (Ando et al., 1983) of Fe (II) and Mn (II) to Fe (III) and Mn (IV) (Ando et al., 1983; Otte et al., 1989). Although the deposition of Fe (III) in the rhizosphere prevents excessive Fe (III) uptake by plants (Bartlett, 1961; Green and Etherington, 1977), formation of Fe- and Mn hydroxides on the roots adsorb cations and prevents nutrient uptake, leading to nutrient deficiency in plants (Otte et al., 1989). The Fe plague also enhances Zn uptake but acts as a barrier in the presence of excess Fe on the root surface (Otte et al., 1989).

Based on the finding of similar studies available in the literature, the observations made on the farm site, the analysed water sample and plant tissue data, and the results obtained from the two bioassay experiments carried out, this study concludes that Fe was responsible for the death of the vegetation (Figure 1) due to the formation of Fe-complex plagues, acting either as barriers to nutrient uptake or enhancers of excessive uptake of potentially toxic metals, or both (Otte et al., 1989), which resulted in the pollution (Figure 1). This study's conclusion is similar to that of (Appleyard et al., 2004b; Haling et al., 2011; Hinwood et al., 2006) where high concentrations of Fe coupled Al have been reported to have potential impacts on ecological health, hence environmental problems. Although the initial water and soil pH measurements indicated the absence of acidity, there is a possibility that the toxic elements accumulated in the drains due to the disturbances of ASS on the farmland soil (Boylen, 1996; Starr, 1996).

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