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The effect of copper stress on inter-trophic relationships in a model tri-trophic food chain.

This thesis is submitted as part of the requirement for a Masters of Research.

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July 2017

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Abstract

Soil fertility and management are paramount in ensuring food security for the growing populations. The use of agri-chemical and products containing heavy metals inadvertently threaten both food security and the surrounding ecosystems from contamination, loss of productivity or ecosystem service. In the present study a series of experiments on the toxic and adaptive responses of wheat plants to copper-induced stress were conducted to establish the effects of different levels of Cu (0 – 200 mg kg^{-1}) on growth, nutrient levels and the total plant proteins of wheat seedlings (*Triticum aestivum*) using pot experiments. A tri-trophic food chain soil → plant → herbivore → predator was established as plants were infested with grain aphids (*Sitobion avenae*) which were subsequently fed to predatory ladybirds (*Adalia bipunctata*). Multiple measurements were conducted which deduced that Cu was taken up from soil into the plant tissues accumulating in the shoot and ear. The rate of growth and flag leaf length were affected by levels of Cu in the soil but total plant mass and ear weights were not. Wheat shoots and ears were analysed for N (crude protein) P, K and it was found that the levels of Cu in soils affected the levels of protein in both the shoot and the ear while the levels of P and K remained unaffected. Total populations of aphid and aphid fecundity appeared to be unaffected by the Cu stress-induced plants and no significant relationships between levels of N in plant tissues or flag leaf length were found. Ladybirds also appeared to be unaffected by the levels of Cu in soils as consumption rate or change in mass between the treatments was not significant. While the present study does not support a critical threshold for Cu levels in agricultural soils it can conclude that biological control methods are unaffected by levels of Cu in the soil.

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1.0 Introduction

"The nation that destroys its soil destroys itself"

President Franklin D. Roosevelt wrote on February 26 1937 in a letter to all state Governors on a uniform soil conservation law.

Presented here is a novel research project which aims to quantify plant productivity in the presences of a heavy metal and the affect this has on inter-trophic relationships in a model tri-trophic food chain which would be found in argi-ecosystems globally. It considers situations where ecological interactions are indirectly affected rather than directly affected by heavy metal pollution whereby the heavy metal itself is not transferred from one trophic level to the next but there remains a trophic cascade of effects within the ecosystem.

This research aims to address this gap in the knowledge by establishing a need for more research in to the indirect effects of heavy metal contamination and other forms of soil pollution on ecosystem functioning in agri-ecosystems.

1.1 Background

The United Nations declared 2015 the International year of soil and commissioned the first major assessment of soils on a global scale. The report emphasised that soils were a natural resource and should be sustainably managed in order to provide the foundation of global food security, yet the report highlighted that a third of the world's soils were moderately to highly degraded (FAO 2015). Physical and chemical soil degradation results in structural changes which can lead to erosion, nutrient depletion, salinity and the accumulation of contaminants such as heavy metals (Rojas et al. 2016; Liu et al. 2014).

The decline of soil productivity due to degradation threatens food security and has detrimental effects on the environment. There are also the financial implications from the reductions in food production, impacting both local and global economies (FAO 2015). Soil fertility is important in agriculture as it directly affects productivity and yield (Bellacasa 2015). Global financial markets are dependent on production. Any drop in agricultural production can cause massive shifts in international prices (Dorash 2009). With higher commercial demands for increased yield, agricultural intensification and chemical fertilization have become top priorities in all agricultural industries (Bellacasa 2015). The use of agrichemical such as fertilisers, herbicides and pesticides all contribute to the accumulations of heavy metals and therefore degradation of agricultural soils (Tscharntke et al. 2002).

Any reduction in food production results in financial loss for producers impacting both local and global economies (FAO 2015). In developing countries 30% - 50% of the total household expenditure can account for the major food staples such as rice, wheat or maize (Dorash 2009). Wheat has been identified as a key crop for global food security (Flood 2010). Currently direct consumption provides 35% of calorific intake for people in developing countries and more than 74% of the calorific intake of people in developed countries (Shifeaw et al. 2013). The global demand for wheat is increasing with population making wheat one of the largest global food commodities (Dorash 2009; Shifeaw et al. 2013). Along with the demand for wheat and other cereal crops is the demand for agri-chemicals that contain essential nutrients used to counteract deficiency in the soils to maximise crop production. However these inadvertently add heavy metals to soils, which can accumulate and degrade the soils (He et al. 2005).

The term heavy metal can be used to describe any transitional metal with an atomic mass over 20; these metallic elements often have a toxic effect on plants and animals even at low concentrations (Rascio & Navar-Izzo 2011). Hence, the occurrence of heavy metals in soils is an increasing point of concern, both agriculturally and ecologically (FAO 2015). The inadvertent accumulation of heavy metals through industrial pollution and the use of agricultural chemicals, even at safe levels, may be affecting species at the foundation of agri-ecosystems (Zhuang et al. 2009).

Many heavy metals are essential for a variety of biochemical and physiological mechanisms in plants (Mudgal et al. 2010), and are crucial to plant metabolism and functioning. Moreover plant uptake is the principal transference pathway whereby metals enter ecological systems (Page et al. 2014). Many types of pollution can disturb tropic interactions depending on the physiology of a species to store, metabolise or excrete the contaminant; this can cause changes in the functioning and interactions within an ecosystem (Butler and Trumble 2008). It is understood that bio-magnification of trace metals effects soil-plant-arthropod pathways thus may have an ecological impact (Weibull et al. 2003). Much of the current research surrounding the presents of heavy metals in the environment primarily focuses on aquatic ecosystems with a lesser amount focused on agriculture and other terrestrial systems (Green & Walmsley 2013).

In agri-ecosystems the main focus has been on heavy metals entering the food chain and the movement of these heavy metals through the various trophic levels. Many studies have quantified the transfer and accumulation of heavy metals within plant tissues and the subsequent transference into the human (Peralta-Videa et al. 2009) and animal (Weibull et al. 2003) food chains to assess to possible risks of toxicity.

Although many studies acknowledge that the pollution of soils has the potential to have serious ecological impacts (Green & Walmsley 2013) there is little evidence of the indirect effects of heavy metals contamination on ecosystem functioning. For example, it is well established that air pollution can change the nature of plant-herbivore relationships (Butler & Trumble 2008), but little is known about the indirect effects of heavy metal pollution. Potentially, the presences of heavy plants in the soil could alter the dynamics of the plant-herbivore-predator relationships. The gap in knowledge could potentially have serious consequences on ecosystem services affecting global food security and quality; species distribution and diversity; and biological control (Chaplin et al. 2000; He et al. 2005; Rantala & Roff 2005; Skirvin et al. 1997). It is important to safeguard ecosystem functioning within an agri-ecosystem as it directly effects of biodiversity through multi-trophic levels which dictate many anthropogenic methods and processes implemented in agriculture.

Plant uptake is the principal transference pathway for heavy metals entering the food chain (Page et al. 2014). Therefore plants are ultimately responsible for the movement of heavy metals from the soil into ecosystems (Nagajyoti et al. 2010) following the source-receptor-pathway. Plants differ considerably in the ability to up-take, redistribute and store heavy metals, factors which are all dependant on the species of plant, developmental stage and the availability of other essential nutrients and other contaminants (Page et al. 2014). The indirect effects of heavy metal pollution are is often overlooked as it is difficult to quantify.

There has been little consideration to situations where ecological interactions are affected by heavy metal pollution where the heavy metal itself is not transferred from one trophic level to the next, i.e. where there is a trophic cascade of effects, but no transfer of the metal. Consequently, there is a fundamental gap in the knowledge regarding the ecological effects of trace metal contamination, one which could have serious consequences for important ecosystem services such as food production and biological control. This research aims to address this gap in the knowledge by investigating the indirect effects of heavy metal pollution on ecosystem functioning in an agri-ecosystem.

1.2 Literature review

1.2.1 Agriculture

Farming practices differ throughout the world; each management strategy is dependent on the farmer's knowledge, crop type, economics and quality of soils (Liu et al. 2014). There are many arguments regarding how best to manage agricultural land with regards to the optimal strategy to maximise production (Tscharntke et al. 2002). Increasingly, farmland is becoming more intensively managed with a growing reliance on agri-chemicals, many of which contain heavy metals, especially in the cultivation of cereal crops (Tscharntke et al. 2002; Liu et al. 2015).

Agricultural land is viewed in terms of productivity rather than as an agri-ecosystem (Savary et al. 2012; Weibull et al. 2003) and is extensively managed only to increase agricultural production (Tscharntke et al. 2002). For example, much of central Europe is dominated by agricultural land, with little space being set aside for conservation (Tscharntke et al. 2002). The demands of an increasing human population has put additional pressures on agriculture, not only in terms of intensive food production but also from land repurposing due to urban spread and industry growth. Between 1990 – 2000, 70.8% of land repurposing in the EU was from agricultural land (FAO 2015). Agricultural intensification, loss of habitat , decline of mixed farming methods, the increased use of agri-chemicals along with climate change have been cited as contributing factors in the reduction of farmland biodiversity (RSPB 2016; Lohaus et al. 2013).

Heavy metals enter agricultural ecosystems via natural and anthropogenic processes. Natural inputs of heavy metals are generally low, occurring through the weathering and breakdown of soil parent material (Brady & Weil 2008). There are many anthropogenic activities that increase the presence of heavy metals in the environment, such as industrial processes, mining, motorised traffic, and military activities (Butler & Trumble 2008). In agriculture the majority of surplus heavy metals are from use of agricultural chemicals and applications (He et al. 2005). Recycled waste materials, slurry and meat and bone meal are often applied to agricultural land to increase organic matter content or nutrient levels in the soils, many of these also contain heavy metals (Khan et al. 2015). However, the main input of heavy metal additions to agricultural soils is from the recycling of sewage sludge (Nicholson & Chambers 2008).

The management of agricultural land directly effects of rate of heavy metal accumulation; the additional pressure of maximum yield production from smaller areas increases the application of agri-chemicals (Khan et al. 2015). Intensive of mono-cropping also significantly escalates the use of argi-chemicals resulting in elevated levels of trace elements and other contaminants (Jojas et al. 2016). Organic and conventional farming practices differ in the use of several management strategies (Chirinda et al. 2010). However, both practices essentially use agri-products such as soil fertilisers, liming agents, fungicides, pesticides and herbicides, which all commonly contain Copper (Cu), Zinc (Zn), Iron (Fe), Magnesium (Mn), or Arsenic (As) as active ingredients (He et al. 2005). Over time, these heavy metals can accumulate (Tscharntke et al. 2002).

Long term applications of any agricultural materials will affect chemical and physical characteristics of the soil, which will ultimately affect the yield (He et al. 2005; Penha et al. 2015). Excessive use of fertilizers, synthetic or mineral based, along with other agri-chemicals often have negative effects on the surrounding environments through soil and water transfer (Flood 2010; Liu et al. 2014). The unnecessary use of fertilizers on already fertile soils is common practice in many countries to increase yield and productivity (Chirinda et al. 2010; Liu et al. 2014). Thus, increasing the accumulation of contaminants and trace elements, such as heavy metals in soils, this results in a reduction in soil fertility, and the soils physical and structural degradation (Liu et al. 2014).

Organic farming systems often use composted materials to fertilise the soil. These materials are derived from vegetable and animal waste and uban/agro industrial waste (Zaccone et al. 2010). Conventional farming methods also include applications of slurry products presenting both agricultural and waste disposal benefits (Penha et al. 2015). Generally, livestock manure is high in heavy metals because assimilation from livestock feed is low (Guan et al. 2001). The use of recycled and waste products (Table 1.1) is regulated by UK legislation and governed by the Environment Agency to ensure minimal environmental impact (EC 2014; Nicholson et al. 2006). During 1996/7 480000 tonnes of bio-solids were applied to 73000 hectares of agricultural land in the UK (Nicholson et al. 2006).

Table 1.1 Concentrations of heavy metals in different agricultural inputs (Adapted from Nicholson & Chambers 2008)

	mg kg^{-1}						
Source	Zn	Cu	Ni	Pb	Cd	Cr	As
Sewage Sludge	802	565	59	221	3.4	163	6
Cattle Slurry	170	45	6	7	0.3	6	2
Pig slurry	650	470	14	8	0.4	7	2
Cattle Farm Yard Manure	68	16	2.8	2.4	0.2	2	1.2
Pig Farm Yard Manure	240	168	5.2	3.2	0.2	2.4	0.8
Inorganic Nitrogen	14	10	1.4	4.6	0.9	3.4	0.9
Inorganic Phosphate	654	94	63	10.5	30.6	319	22
Inorganic Potash	8	6	0.8	2.7	0.5	2	0.5
Lime	11	2	5.1	2	0.3	6	*
Compost	182	46	17	96	0.06	19.8	*

*No data available

The Environment Agency published the UK soil and herbage pollutant survey (EA 2007) which assessed the concentrations of heavy metals in UK soils (Table 1.2), this report highlighted the range of accumulations of heavy metals in soils across the UK. To avoid excessive heavy metals in soils the Contaminated Land Exposure Assessment sets out Soil Guideline Values for the maximum permissible soil concentrations of some heavy metals (EA 2009). However these levels do not provide values for agricultural land focussing on soils in residential and commercial setting or land used for allotments it also does not consider any values for Cu or Zn in any setting. European Community directive 86/278/EEC does set limits on the concentrations of Cu and Zn, along with Cd, Cr, Hg, Ni and Pb that are permissible in agricultural soils, but only when sewage sludge is applied to soil. Consequently, the accumulation of heavy metals in agricultural soils is poorly controlled.

Table 1.2 The Concentration of metal in UK soil types (mg kg^{-1}) Produced by the UK Soil and Herbage survey (EA 2007).

	Rural soils mg kg^{-1}		Urban soils mg kg^{-1}	
Metal	UK Range	UK Mean	UK Range	UK Mean
Cu	2.27 - 96.7	20.64	8.27 - 181	42.50
Mn	10 - 12200	612.00	98.3 - 2100	502.00
Ni	1.16 - 216	21.10	7.07 - 102	28.50
Pb	2.6 - 713	52.60	8.6 - 387	110.00

Zn	2.63 - 442	81.30	35.1 521	121.50

Heavy metals outputs from natural and anthropogenic sources can also become deposited on to soils from the atmosphere (Liu et al. 2015). Zn, Cu and Pb are readily translocated from atmospheric deposition. But the conditions of release into the atmosphere make it difficult to predict the original source of the quantity (Nicholson et al. 2006). Atmospheric deposition accounted for 2485, 638, 180, 611, 22, 84 t of Zn, Cu, Ni, Pb, Cd, Cr and As, respectively (Nicholson & Chambers 2008) to be added to agricultural soils in England and Wales in 2004. Contaminants can also be contained in irrigation and sewage water used in the production of many crops (Liu et al. 2015) or from waste water or runoff from industrialised areas (Patel et al. 2008). Sewage sludge applications provide a pathway for a number of potentially toxic elements to enter agricultural soils, hence the control of this substance by EU directives.

In total 5934, 1648, 371, 18960, 39, 383, 80 t of Zn, Cu, Ni, Pb, Cd, Cr and As respectively (Nicholson & Chambers 2008) were added to agricultural soils in England and Wales in 2004 from a various anthropogenic sources. Acute metal contamination in soils is infrequent and so is often overlooked even though long-term effects can be severe (Nica et al. 2012).

1.2.2 Food security

Food security ensures food production and availability through agronomic management of soil resources (Rojas et al. 2016). As the global population increases, so does the demand for food, increasing the pressure on agriculture, industry and urbanisation (Patel et al. 2008). The global population is predicted to reach nine billion by 2050 (EC 2014) and it is suggested that major changes in agricultural management will be required to increase currant food production by 60% to meet future demands (Rojas et al. 2016).

Food security is dependent on soil fertility. Fertilizer use, land and water management are vital to crop yield performance (Liu et al. 2014). However, it is also essential to minimise environmental impact of food production to sustain and maintain natural resources, raw materials and clean water systems (EC 2014). There is an increased reliance on metal-containing substances in agriculture, especially in the cultivation of cereal crops to maximise yields. Such substances include inorganic fertilisers, inorganic fertilisers, liming agents, fungicides, pesticides and herbicides which commonly contain Cu, Zn, Fe, Mn, and As (He et al. 2005).

This increasing global demand for food means that contaminated land is being used for agricultural use and requires extensive agricultural applications to achieve the desired plant productivity, increasing the risk to both human health and agri-ecosystem functioning (Ran et al. 2016). There is a need for the development of agricultural technologies and the generation of more productive crop varieties to ensure crop productivity meets the growing global demand (Hawkins et al. 2013). Soil sciences are becoming more pivotal in the future of food security as it incorporate social, poverty alleviation, land degradation and the provision of environmental services on a global platform (Sanchez et al. 2003).

1.2.3 Soil

“The protection of soils is and should be a principal objective of environmental policy. Soils are a finite, increasingly scarce and non-renewable resource with varying biological, chemical and physical properties. These should be protected and preserved in order to maintain the soils important ecological functions. “

(European Soils Bureau Scientific Committee 1999)

Soils are the bases of all terrestrial food chains (O’Hare 1988) soil characteristics are essential factors in food production; it is generally accepted that soil health is required for the optimum growth of most plants. The evaluation of soil characteristics and physiochemical properties such as heavy metal bioavailability and climatic conditions are becoming increasingly important in order to maximise yield in agricultural settings (Khan et al. 2015; Nica et al. 2012). The agricultural approach to soil health primarily focuses on the productive functions of soils rather than considering the role of soils within the wider agri-ecosystem (Sanchez et al. 2003). This leaves soils vulnerable to degradation from accumulation of contaminants and ignores the importance of ecological interactions such as biological control, often associated with crop production.

Accumulation of heavy metals is generally confined to the top soil (Guo et al. 2013) and is less visible than other types of pollution so is often overlooked even though long term effects can be severe (Nica eta l 2012). Heavy metals can be present in soils in various forms including soluble and exchangeable ions, oxide complexes, and carbonates or adsorbed to organic materials or clay particles (Guan et al. 2011). Soil chemistry and characteristics strongly influence the availability of heavy metals to plants and the subsequent movement through the trophic levels.

Many trace elements, including heavy metals, become more soluble in acidic conditions (Brady & Weil 2008). Organic matter and clay particles carry a negative charge over the outer surface allowing for the adsorption of cations to bind to the particles (O'Hare 1988). Cations that are adsorbed to soil particles are unavailable for plant uptake. However, cations can be displaced with H^+ ions contained in the soil solute. An increase in H^+ ions will cause the metal ions to disassociate from the soil particles and become contained within the soil solute, thus allowing them to be taken up by plants (O'Hare 1988; Campbell et al. 2008). Thus, pH is considered to be the most important factor in heavy metal mobility and bioavailability (Zeng et al. 2011), the lower the pH the more acid-extractable metals become (Guan et al. 2011). The ideal pH for agricultural soil is pH 6.5, this is often maintained through the use of agricultural products (O’Hare 1988), which can further sources of heavy metals.

The organic matter increases the water retention and increases the soil cation exchange capacity of soil allowing for the sorption and desorption of ions and other molecules (Bohn et al. 2001). This releases both plant nutrients and contaminants into the solute making them available for plant uptake (Schumacher 2002). These changes the soil chemistry affect the soil flora which can then change the nutrient cycling processes, which in turn alters the nutrient and contaminant availability within the soil solute (Khan et al. 2015; Zeng et al. 2011; Winder et al. 1999).

Cu, Zn, Cd and Pb are the most common heavy metals that pollute the environment (Nica et al. 2012; Son et al. 2016). Cu contamination is agro-ecosystems can occur due to the use of high Cu fungicides/bactericides (e.g. Bordeaux mixture, copper oxychloride) and animal manures, particularly from pigs that are fed Cu as a growth promoter. Cu is not readily accumulated in humans or other mammals, so research in to the effects of copper transferred through food chains has not been considered a priority (Fernandes & Henriques 1991). Although the quantity of Cu entering the soil in England and Wales is much lower than in many other European countries (Nicholson & Chambers 2008), Cu levels in soils can become problematic when Cu is contained in agri-chemicals that are applied to the soil in agricultural practices (Fernandes & Henriques 1991).

1.2.4 Plants

In agricultural crops, maximum yield is dependent on genetic variance, agricultural inputs and environmental conditions (Spychay-Fabisiak et al. 2014; Hawkins et al. 2013). Nutrient depletion and stress factors prevent the plants from reaching full yield (Tuteja at al 2011). Reduction in soil nutrients occurs when the nutrient output exceeds nutrient inputs; this can occur over varying timescales and can have overwhelming limitations to plant growth (Sanchez et al. 2003). The removal of crops during harvesting is the biggest output of trace elements from the agro-ecosystem (He et al. 2005) as it removes both nutrients and organic matter. Other stress factors include pollution, temperature extremes, water availability, and ion toxicity, including salinity and heavy metals (Tuteja et al. 2011).

The symptoms of stress, deficiency or toxicity of trace elements can often be mistaken for drought, disease, or insect damage which may not be obvious in developing or mature plant tissues (He et al. 2005). Trace elements that are required for growth can have phytotoxic effects to plants when they are absorbed above a certain threshold (Peralta-Videa et al. 2009) disturbing metabolic processes that may cause oxidative damage to tissues, decrease growth and/or development of the plant (Panda et al. 2003). This can lower the nutritional value by reducing the production of carbohydrates, starches and lipids, thereby altering the development of the plant, with concomitant effects at a primary trophic level (Lepp 1985; Lanaras et al. 1993).

Plants absorb both essential and non-essential levels of heavy metals from soils through selective uptake of ions by the roots either down concentration gradient, by diffusion or through active transport (Peralta-Videa et al. 2009). The structure of the root and root hairs maximise surface area for the absorption of water and solutes (Campbell 2008). The anatomical characteristics of the root determine the nutrient and contaminant uptake, and therefore the growth rate of the plant (Ovecka & Takac 2014).

Molecules, including heavy metals, enter into the epidermis by crossing through the cell wall of the root hair. Water molecules diffuse through the polysaccharide rich cell wall readily, often with dissolved solutes (Holbrook 2011). Solutes that are unable to diffuse through the cell wall are actively transported across the plasma membrane by transport membrane proteins (Campbell 2008). Any substance taken up by the roots has to pass through the root cortex and past the casparian strip before reaching the phloem and xylem vessels within the stele (Campbell 2008; Saxena & Misra 2010). There are three pathways that cross from the epidermis to the stele and so into the phloem; apoplastic, symplastic and transmembrane (Ovecka & Takac 2014). These pathways are not fixed, so water and solutes are able to pass between the different routes liberally until the molecules reach the casparian strip, which acts as a barrier to apoplastic transport.

The apoplastic pathway involves the movement of water and solutes through the extracellular matrix within the cell wall (Holbrook 2011). Pores in the cell wall allow molecules to flow along a hydrostatic gradient from cell wall to cell wall (Campbell 2008), resulting in a relatively unregulated passage way (Saxena & Misra 2010). Once the apoplastic pathway reaches the Casparian strip any solutes have to pass through a plasma membrane to gain access to the stele (Campbell et al. 2008). Potentially, this could allow regulation of the solute's transport.

The symplastic pathway involves the transport of molecules through plasmodesmata, a selectively permeable plasma membrane (Campbell 2008), which provides a physical barrier against the entry of molecules into the cell (Ovecka & Takac 2014). The plasmodesmata connect the vacuoles creating a continuum through the root cells. This this means that a molecule only has to pass through one plasma membrane to gain access to the stele (Holbrook 2011).

The transmembrane pathway sees water and solutes moving in and out of cells across multiple plasma membranes (Campbell et al. 2008). This route is energy dependant and involves multiple metal specific and generic metal ion transporter proteins and channels (Saxena & Misra 2010).

1.2.5 Heavy metal uptake in plants.

Contaminants and heavy metals cannot move freely through a plants vascular system, these molecules have to form complexes in order to be transported along the apoplastic, symplastic and transmembrane pathways (Saxena & Mistan 2010). The casparian strip is a non-permeable ring of cells in the endodermis that surround the stele forming a physical barrier between the epidermis and the stele (Holbrook 2011). Water diffuses across the casparian strip by diffusion; transpiration creates the concentration gradient and negative pressure to effectively pull the water into the xylem (Campbell 2008). Solute molecules are transported across the plasma membranes by active transport via transporter proteins. This compartmentalisation enables the plant to retain and therefore separate unwanted molecules in the epidermis in order to protect the rest of the plant tissues (Holbrook 2001).

Plants take up a number of trace elements that have accumulated in the soil (Patel et al. 2008). The rate of nutrients and heavy metals up take by plants is based on the bioavailability of the molecules defined by the soil conditions, the motility and retention of the substance (EA 2007). Not all heavy metals are harmful to plants. Cu, Fe, Mg, Zn, and Ni are micronutrients and so are actively absorbed by plants to enable certain biological functions (Peralta-Videa et al. 2009).

Both the deficiency of essential heavy metals and the excess of essential and non-essential heavy metals can slow growth and development and reduce yields of a plant or crop (Dorrington & Pyatt 1983; Stern 1997). Any uptake in excess of the organism requirements is considered to be a toxic amount, including the essential heavy metals which have many important functions: Cu and Mn are required for photosynthesis and vitamin production, while Ni is used in the synthesis of enzymes and Fe is indispensable for all live organisms (Nagajyoti et al. 2010). It is generally understood that these protective mechanisms require an expenditure of the plants energy and consequently this reduces the plant's growth and productive capabilities (Lepp 1985).

Plants become stressed when grown in elevated levels of heavy metals altering biochemical reactions within the plant (Peralta-Videa et al. 2009). At high concentrations of heavy metals, cell walls become compromised as the cell membranes lose their integrity allowing for the passive flow of heavy metals to become the dominant pathway into the plant cells (Fernandes & Henriques 1991). The detoxification mechanisms that are necessary to protect the plant are active processes and so require energy. Any process that restricts or promotes the elimination or encapsulation of toxins is a metabolically expensive undertaking (Sibly & Calow 1989) which reduces the productivity of a plant resulting in lower plant biomass and changes in the structure of chloroplasts (Pavlovic et al. 2014).

Heavy metal transporters increase the sequestration of heavy metals (Ovecka &Takac 2014). Many transporters have been identified although many associate with heavy metals are thought to be ZIP (Zinc regulated/iron regulated like protein) transporters, which are non-specific (Clemens et al. 2002). These are involved with the uptake and accumulation of both essential and non-essential trace elements, including heavy metals. While cation diffusion facilitators (CDFs) are more selective (Ovecka & Takac 2014) moving heavy metals from cytosol to other cellular compartments (Clemens et al. 2002).

Plants have developed mechanisms to accumulate high levels of metals within their tissues without exhibiting symptoms of toxic stress (Garcia-Salgado et al. 2012). It is this protective mechanism that relies on the production of phytochelatins (PCs) as part of a stress response; PCs are small polypeptides that bind to heavy metal ions in plant tissues (Ovecka & Takac 2014). The heavy metal-phytochelatin complexes prevent interactions between heavy metals and any functioning systems, therefore detoxifying the heavy metals (Yadav 2010). The heavy metal-phytochelatin complexes move through the phloem enabling translocation through plant tissues without deleterious action on the cellular membranes (Yadav 2010).

Cu, Zn and Ni do not compete for transporters as they each have their own. So concentrations of a single metal do not affect the accumulations or uptake of the other metals (Green & Walmsley 2013). Cu uptake by wheat is generally low, even when growing in Cu enriched soils, and is generally restricted to roots with minimal translocation (Guan et al. 2011). In addition within plant tissues Cu accumulates in the root more than other plants tissues (Wang et al. 2013). This prevents translocation through to other plant tissues, which is a common tolerance mechanism in many plants (Fernandes & Henriques 1991).

Copper (Cu) is an essential micronutrient required by plants for optimal growth, development and the synthesis of proteins and enzymes involved in photosynthesis (Tuteja et al. 2011), but excessive amounts can have phytotoxic effects that inhibit these processes (Lanarus et al. 1993). At increased concentrations, Cu becomes toxic to many plants. Prolonged exposure to copper within plant cells degrades the cellular membranes of the cell and the organelles contained within, especial the membranes of chloroplasts (Fernandes & Henriques 1991). Cu toxicity interferes with plant functioning, causing variations in metabolites, changes to plant architecture and, crucially for plant-herbivore interactions, interferes with the production of defence chemicals (Bukovinszky et al. 2008).

Excessive Cu also impedes photosynthetic processes as it inhibits many enzymes that play an active role in the dark phase of photosynthesis (Fernandes & Henriques 1991). Photosynthesis is the basic process of plants to utilise nutrients to synthesise food and is primarily achieved by chloroplasts within the shoot tissues (Jiang et al. 2016). Any reduction in chlorophyll will inhibit photosynthesis and reduce growth and development of a plant.

1.2.6 Wheat

Wheat has been grown in agricultural settlements for the last 8 – 10 thousand years and it can be grown in a variety of climatic conditions (Smith 1998). Modern wheat varieties have been rapidly adopted by both developed and developing countries, with more than 220 million hectares planted annually producing 670 million tons of grain (Shiferaw et al. 2013). It has become an important cereal crop; it is the principle source of energy, protein and dietary fibre for a large proportion of the world's population (Jiang et al. 2014; Liu et al. 2014). It is estimated that wheat contributes around 20% to the total calories and protein dietary requirement worldwide (Shiferaw et al. 2013).

Monocultures, such as cereal crops create high densities of pest-prey species (Green et al. 2010). Aphids are the most serious animal pest of wheat and interactions in the cereal-aphid-predator systems are of great importance in the production of cereal crops and the control of pest species on a global scale (Flood 2010; Losey & Vaughan 2006; Tuteja et al. 2011). Cereal producers rely heavily on pesticides to maximize wheat production (Savary et al. 2012), but many of these agri-chemicals contain Cu, Zn, Fe, Mn, and As (He et al. 2005). Current annual loss of yield due directly to pest and pathogens is estimated to be around 13% (Shifeaw et al. 2013) with indirect losses to predicted much higher (Savary et al. 2012).

Within plant tissues, Cu accumulates in the root more than other plants tissues (Wang et al. 2013). The accumulation and transference of Cu is dependent on the genotype of the plant (Alybayeva et al. 2014; Clemens et al. 2002) and in many plants causes metabolic disturbances and growth inhibition as Cu inhibits a variety of enzymatic and biochemical interactions within plant tissues (Fernandes & Henriques 1991). This can affect the quality of the grain but also make the plant more susceptible to pest damage (Awmack & Leather 2002), potentially altering the plant-herbivore relationship in favour of the pest.

1.2.7 Aphids

Aphids are small and inconspicuous plant sucking insects from the order Homoptera (Dixon 1973). Aphidoidea dominate lower trophic levels within agro-ecosystems and the success and quality of these organisms directly affects all subsequent trophic levels (Green & Walmsley 2013). Individually aphids are quite harmless, but in relatively small numbers they can exploit plants resources, draining nutrients and energy from the plant (Dixon 1973). They are responsible for causing economic loss and damage, through degradation and the transference of plant diseases of crops around the world (Wanlei et al. 2009).

Sitobion avenea, *Metoplohium dirhadum* and *Rhopalosiphum padi* are the major cereal aphids species across Europe (Lohaus et al. 2013), capable of reaching high population densities destroying host crop plants and lowering grain yields (Harmon et al. 2009). In china alone it is estimated that aphids are responsible for up to 40% of yield loss (Xin et al. 2014).

Aphids feed by inserting slender mouthparts, stylets, between cells aided by saliva which breaks down the bonds between cells to reach the phloem tissue where the stylets can pierce the cells to begin feeding. Here the sap is under considerable pressure, which the aphid relies on to force the sap up the fine tube of the stylet (Dixon 1973). The phloem sap has high concentrations of sucrose, but low concentrations of amino acids and other essential nutrients, which presents a nutritional challenge to the aphids, making them sensitive to changes in plant quality (Cristofoletti et al. 2003; Dixon 1973). When the amino acid content of the phloem is at its highest, the size and fecundity of aphids is at its greatest, as concentrations of amino acids declines so does the size and the fecundity of the aphid (Awmack & Leather 2002). Hence, host plant quality is an important parameter influencing aphid populations.

Symbiotic bacteria of the genus *Buchnera* in the aphid gut enables modification of amino acids obtained in the phloem to improve the nutritional quality of the diet (Awmack & Leather 2002). Studies by Ponder et al. (2002) suggested that variations in plant nitrogen availability affect the production of amino acids in phloem sap, which are required for aphid development. This sensitivity to the quality of their host plants (Gorur 2007) makes aphids a good biosensor of plant stress and so can be used to measure the effects on subsequent trophic levels.

Aphids become established on host plants in early spring when food resources for the aphid are at the maximum and predation is low (Dixon 1973). Aphids directly feed on wheat plants from April thought to August and, only severe infestations produce visible symptoms of stress turning the leaves yellow and causing them to senesce prematurely (AHDB 2014). Generally, aphid reproduction is successful between 10^0C and 30^0C, but aphids are known to be extremely sensitive to short-term variations in temperature (Skirvin *et al.* 1997). A female aphid can give birth to live young, which in turn can play host to developing embryos, as females do not require fertilisation. This allows for rapid population expansion (Dixon 1973).

Aphids have complex life cycles, when conditions become less favourable due to overcrowding, declines of plant nutrient levels or seasonal change; aphids can develop into morphed winged adults allowing dispersal (Dixon 1973). Higher temperatures will be favourable to aphid populations as climate change has extended the aphid flight season by 3 days per decade (Morecroft & Speakman 2015). Winged adults are not strong flyers but can migrate long distances relying on wind dispersal to exploit new plant resources, to lay eggs to survive winter and hatch out the following spring (Dixon 1973; Xin et al. 2014). The extension of the flight season will allow for greater distance for dispersal.

As herbivorous arthropods, aphids provide the link between primary producers and upper trophic levels (Winder et al. 1999) via the plant-herbivore-predator pathway (Wanlei et al. 2009). Their soft bodies and sedentary nature (Dixon 1973) make them a primary food source for predatory arthropods in agro-ecosystems (Bilde & Toft. 2001). As aphids are predated by a large number of species, any changes in population numbers will impact predating species in the next trophic level (Gorur 2007; Bukovinszky et al. 2008).

Reproductive efforts, development, size and immune defence are all factors in optimising overall fitness (Rantala & Roff 2005), which is dependent on the nutrient intake (Khan et al. 2015). To maximise nutrient extraction from the phloem the aphid mid-gut had adapted to the high carbohydrate, low amino acid levels of the phloem sap (Cristofoletti et al. 2003; Dixon 1973). The lining of the aphid mid-gut has amino acid binding proteins on the surface of the perimicrovillar membranes, enabling assimilation of the amino acids. Metabolism of amino acids is facilitated by symbiotic bacteria *Buchnera* which are found in the mycetocytes of the haemocoel (Cristofoletti et al. 2003). *Buchnera* play an important role in nitrogen fixation and it is this symbiotic relationship that allows aphids to survive on such restricted diets (Gullan & Cranston 2005).

Aphids are known to thrive in polluted environments; studies have shown that aphids on host plants subjected to increased SO_2 and NO_2 or O_3 have increased growth rates and greater population increases (Butler and Trumble 2008). These studies suggested that air pollutants change the nutritional composition in the host plants, increasing the availability of amino acids and carbohydrates to insects. Adult aphids are sensitive to nutrient availability and chemical changes in plants either from defence mechanisms or sequestration of heavy metals (Dixon 1973: Bilde & Toft. 2001) as it changes the nutritional composition in the host plant. Cu is known to have an effect on many of the biological processes within plant that affect both the formation of defence mechanisms and sequestration (Clemens et al.   2002; Tuteja et al. 2011). Consequently, it is difficult to predict the effect of Cu on plant-herbivore interactions due to pollution, as the fitness of herbivorous insects could be beneficially affected, negatively affected or be unaffected depending on the balance of the effects in the host plants.

Aphids are known not to accumulate Cu in body tissues; instead any excess of Cu ingested gets excreted in honeydew (Crawford et al. 1995). Therefore, any effects in aphids will be due to changes in plant nutrient levels and not Cu treatments.

1.2.8 Coccinellids

Coccinellids (ladybirds) belong in the order Coleoptera or beetles, easily recognised for their dome shaped shell and spots. The bright colours of the aposematic colouration of a ladybird are to signal warning of toxicity to predators. When threatened adult ladybird will release defence chemicals, alkaloids, from the joints in their legs whereas larvae have the ability to ooze these chemicals from their abdomen (Dixon 2000). Despite this ladybirds are and important food source for a number of species, including other ladybirds and many birds (Dixon 2000; RSPB 2016).

Ladybirds are polyphagous predators adapted to exploit aphid populations: large body size, high ferocity, high predation efficiency, rapid development and high fecundity mean they are valued biological control agents within an agro-ecosystem (Vandereycken et al. 2013). Over a life time a single ladybird may consume as many as 5000 aphids, with an adult able to consume up to 50 aphids per day (Dixon 2000). Studies show that aphids have poor prey value to a number of arthropods, including ladybirds, and generalist predators require a mixed diet that increases nutrition and improves fitness (Bilde & Toft. 2001). Optimal foraging theory predicts that low quality prey will be rejected if a higher quality prey is available (Skirvin et al. 1997). This means that if aphid quality is reduced due poor nutrition cascading from the host plant, then predation of aphids by ladybirds could be reduced if other prey is available.

Ladybirds remain the most abundant predators within cereal and vegetable crops despite competition from other species (Vandereycken et al. 2013). Retention of ladybirds with an agro-ecosystem is determined by both food availability and quality (Ives et al. 1993). Ladybirds feed on aphids through the summer months to enable them to build up energy reserves for overwintering (Walker et al. 1998). Ladybirds exploit aphid populations as they are an abundant food resource, easily predated for both adults and larvae increasing the fitness and overall success of ladybird populations (Omkar 2014). Predation rate is determined by hunger state, encounter rate and feeding preference of the individual (Bilde & Toft). Similar to many arthropods, ladybirds have a voracious appetite making them ideal biological control agents (Gonzalez et al. 2016. However, ladybirds are mobile predators in the adult form and can emigrate from fields when aphid numbers fall or are of poor quality (Ives et al. 1993).

Ladybirds exploit aphid populations throughout all their life stages (Dixon 2000), with young larvae maximising their fitness by focusing feeding in areas with high densities of young aphids which are easily predated (Hemptinne et al. 2000). Both adult and larval ladybirds are attracted to aphid clusters by smell through either the alarm pheromones released by aphids or the feeding signals given off by feeding adults (Hemptinne et al. 2000).

The life history of the ladybird is governed by the nutritional quality of its diet (Walker et al. 1998). Declines in fitness can be described as significant reductions in survival, longevity, fecundity, size and increased development times (Butler & Trumble 2008). Larvae are less mobile than adults, so are more vulnerable to the effects of prey species feeding from compromised host plants (Walker et al. 1998). The reduction of larval development during periods of low/poor prey availability indicates that there is a trade-off between survival and reproductive life strategies favouring survival over reproduction (Omkar 2014).

Larger individuals require a higher intake of food to fulfil the higher energy requirements associated with a larger size (Omkar 2014). Aphid-only diets, which are nutritionally poor, can results in lower egg laying potential for many arthropod species, including ladybirds (Bilde & Toft. 2001). Even once reproduction and egg laying has ceased, ladybirds will continue and to consume prey at a lower rate to sustain energy levels for maintenance and survival (Butler & Trumble 2008).

As ladybirds are sensitive to the quality of the available diet (Bilde & Toft 2001;Green et al. 2010; Gonzalez et al. 2016; Omkar 2014) it is likely ladybirds will be affected by any changes in the quality of their prey stemming from changes in the host plant (Bilde & Toft. 2001; Walker et al. 1998). Ladybirds appear to be able to tolerate higher levels of Cu compared to other arthropod species (Bilde & Toft. 2001). As a consequence of this, any changes in the nutritional quality of the aphids should affect the survival of the ladybird even in the absence of Cu toxicity affecting the ladybird.

1.2.9 Ecosystem services

Humans have always had an impact on their environment (Chaplin et al. 2000). Agriculture, industry and rural economic developments results in environmental alterations that change ecosystem equilibrium (Brunetti et al. 2012). Any changes to the environment will alter species diversity, which will have a functional impact on ecosystem processes (Chaplin et al. 2000). Abiotic features of a habitat influence biotic interactions as quality of an area will affect the community structure (Tscharntke et al. 2002). Ecosystems withstand and coevolve with many abiotic and biotic disturbances, but the rate and impact of these disturbances could potentially effect ecosystem regeneration and resilience (Coulson & Joyce 2006).

Monocultures create high densities of pest-prey species and as there is a lack of alternative food sources, predators are at particular risk from pollution (Green et al. 2010). Moreover although monocultures may increase the populations of specialised pest species, it may reduce the habitat quality for more generalist predator species (Wanlei et al. 2009), that often require a more mosaic landscape in order to complete their life cycles (Bengtsson et al. 2005) or the provision of adequate nutrients in food resources to sustain larval growth and development (Awmack & Leather 2002). Many predator species depend on resource found in non-crop habitat, such as alterative prey, pollen and nectar and over wintering habitats (Tscharntke et al. 2002). Furthermore, there is evidence that polyphagous insect populations are in decline through the use of pesticides aimed at controlling pest species (Smith et al. 2009). Improving the quality of the habitat for predator species may reduce the need for herbicides and therefore reduces that accumulation of contaminants in the soil.

Biological control is a very important ecosystem service. Integrated pest management, including biological control, is an integral part of sustainable crop production; insecticides are becoming increasingly inefficient in reducing pest species populations so the use of using predator species as a method of biological control is of increasing importance (AHDB 2014).

Sustainable agriculture needs to integrate the use of biological pest management species to allow natural enemies of aphids to provide top-down control of populations. However, the direct and indirect effects of soil pollution through the food chain may have bottom up effects that affect the efficacy of this biological control. This could either reflect an increased effectiveness due to lowered fitness of the prey or decreased effectiveness due to lowered prey quality.

2.0 Present study

This study utilises a model food chain to investigate the effect of a Cu on plant-herbivore and herbivore-predator interactions. Cu is a common agricultural pollutant that is not readily transferred through the soil-plant-aphid food chain. Consequently, any noted effect will not be due to direct toxicity of Cu, but due to disruption of plant or aphid metabolism. The in-direct effect of metal pollution on trophic levels, beyond the primary consumer has been ill-considered, but required elucidation if sustainable agriculture is to be realised.

2.1 Aims

The aim of the present study is to determine the effect of soil copper contamination on plant functioning and nutritional content and subsequent effects on plant-herbivore and herbivore-predator interactions. The research aims to investigate the changes in plant health by measuring the rate of growth, nutritional composition, and copper in the plant to determine what effect this has on plant-herbivore-predator food-chain in order to determine if soil contamination has multi-trophic effects other than transfer through the food-chain.

2.2 Objectives

1. To determine the effect of Cu stress on host plants.

2. To determine if plant stress affects aphid fitness, i.e. fecundity and population biomass.

3. To investigate if effects on the aphid population translate into altered predation by a specialist predator.

3.0 Materials and Methods

3.1 Introduction

The scope for this research utilises the fact that soil-plant aphid system is easily manipulated and has proved to be a useful system for studying the effect of pollutants, including heavy metal, on the interactions between trophic levels. Although many studies largely focus on the transference and accumulation of heavy metals within plant tissues and the subsequent transference, at present we do not know if metals can affect this relationship without transfer, but the consequences may be profound if this is being ignored.

The methods for this research have been designed to investigate direct and indirect consequences of the presence of Cu in an agriculturally relevant soil-plant-herbivore-predator system modelled through a pot trial. Pot trials were conducted in a greenhouse to reduce the effect of variables affecting the system and allow to the experiment to meet time constraints. Wheat (*Tricum aestivum* L. cv. Tybalt) was grown in Cu amended soil; aphids (*Sitobion avenea Fab.*) were then added and monitored before harvesting for use in a feeding trials using two spotted ladybirds (*Adalia bipunctata L.*).

3.2 Soil

A bulk sample (~90 kg) was taken from the Ap horizon of a luvisol soil located in an agricultural field, which was situated near West Chaldon, Dorset, UK. The soil sample was standardised by being thoroughly homogenised by repeated mixing before being passed through a 5mm mesh to remove any stones or large objects prior to use as the substrate for all the pot based experiments.

Cu treatment

The soil was then air dried for 48 hours before being divided into 5 x 15kg sub-samples. Each sub-sample was randomly allocated a treatment of copper sulphate solutions (10 mg mL^{-1}) to increase the soil Cu concentration by 0, 25, 50, 100, 200 mg kg^{-1} (Table 3.1). Distilled water was used to ensure each soil sample received an equal volume of liquid. After the addition of $CuSO_4$ and/or H_20, the soil was again homogenised by repeated mixing.

Table 3.1 Additions of $CuSO_4$ and H_20 added to treat soil samples with Cu.

Treatment	Control	25 mg kg $^{-1}$	50 mg kg $^{-1}$	100 mg kg $^{-1}$	200 mg kg $^{-1}$
Volume of 10 mg mL^{-1} $CuSO_4$ (ml)	0	30	60	120	240
Volume of H_2O (ml)	300	270	240	180	60
Total Volume (ml)	300	300	300	300	300

The amended soil was then left in heavy duty plastic sacks to for 6 months to allow the added Cu to reach equilibrium with the solid phase of the soil (McLaren & Clucas 2001). Each sample was turned twice during this time to ensure homogeneity was maintained.

3.3 Pot trials

Wheat was grown in soil treated with varying concentrations of Cu. It is assumed that a plant growing in heavy metal free conditions would reach maximum growth potential whereas the growth and development of heavy metal stressed plants would be hindered. The reduction in plant productivity would affect the success of the aphid populations in both size and fecundity, which in turn will affect the success of the predator species.

The amended soils were used to fill 5 replicate 5L pots. Each pot was then seeded with spring wheat to the rate equivalent of 400 kg ha^{-1}, (circa

20 seeds per pot) at a depth of approximately 2.3cm deep. Wheat variety used was *Triticum aestivum* L. cv. Tybalt, a spring wheat variety with high yield and high disease resistance (HGCA 2013).

The freely draining pots were then randomly placed in a block design in a greenhouse to minimise any environmental bias (Figure 3.2). Randomisation was achieved by drawing lots (Samuels 1989).

25E	200E	25C	Cont B	50D
50A	Cont D	Cont C	25B	100A
100E	100D	50C	50E	200D
100B	200B	Cont A	200C	25A
100C	Cont E	50B	200A	25D

Figure 3.2 Randomised block lay out of treatments in the green house. Numbers refer to treatments mg kg^{-1} (dry weight) soil, letter to the replicate of that treatment.

Figure 3.3 initial set of pots in their randomised block.

Conditions in the greenhouse were maintained as close as possible to a day: night ratio of 16:8 h using high pressure sodium lamps to augment natural daylight (mean light intensity700 μmol m^{-1} s^{-1}) and ranging between 20-25 ^{o}C in temperature using electric heaters and automatic roof vents to alter temperature.

Each pot trial was watered on demand with distilled water. A 7:7:7 NPK fertiliser was applied at a rate equivalent to 187 kg ha^{-1} in the pot trail on day 41 to ensure the wheat plants received fertiliser at a rate consistent with agricultural practice. Development and rate of growth was recorded every 3-4 d for the following 70 d.

Pot trails were maintained and continued to be watered for 28 d during the aphid trials. On day 98 of the experiment, 5 random flag leaves were measured and after the aphids had been removed the total biomass of wheat in each of the pot was recorded.

3.4 Arthropod cultures

All arthropods used in the present study were taken from laboratory cultures. Grain aphid (*Sitobion avenae*) cultures were started from specimens obtained from Rothampsted Research, Harpenden, UK. Several generations (>5) elapsed before specimens from the cultures were used in the experiments.

Ladybirds (*Adalia bipunctata*) cultures were obtained from Bioline AgroSciences, Little Clacton, UK and were used immediately in the feeding trail.

3.4.1 Aphids

When the wheat plants reached decimal growth stage 37-51, they were manually infested with 50 aphids. This is coincides with the rapid expansion phase of cereal growth when the crop canopy is rapidly expanding and the wheat plant has maximum nitrogen requirements (GRDC 2005).

Each individual pot was covered with a net to prevent transfer of aphids between treatments or the infestation of non-laboratory cultures. Aphids were left to colonise each of the pots for 28 days before being harvested.

On day 95 of the experiment 3 individual aphids from each pot treatment were isolated in clip cages to record reproduction rates of the course of 3 days (Figure 3.4).

Figure 3.4 Clip cages used to isolate single aphids to determine numbers of nymphs produced.

On day 98 of the pot trail experiment aphids were manually harvested from each plant leaf using a fine paint brush. The fresh mass of the aphid populations from each pot was then measured and the aphids collected from each pot were stored in individually at -80^{o}C until used in the ladybird feeding trial or chemical analysis.

3.4.2 Ladybirds

The ladybird feeding trial took place in petri dishes and was based on the method reported by Green et al. (2010). 25 ladybirds were starved for 72 h before being placed in 9cm petri dishes along with damp pieces of filter paper to maintain humidity. The ladybirds were then kept in a controlled environment cabinet set to a 16:8 day night cycle at 24^{0}C.

Each ladybird was assigned a treatment at random before the feeding trial then and offered a 10mg sub-sample of aphids harvested from one pot from that treatment. Ladybirds were fed each day and the previous day's debris was removed from the petri dish and weighed. The difference between the amount given and the amount of debris was considered to be the aphid consumption rate. The consumption rate was recorded daily for 4 d. Fresh mass of ladybirds was recorded at the start and end of the feeding trial.

3.5 Chemical analysis

Soil preparation.

The soil substrate from treatment was homogenised and air-dried before being passed through a 2mm sieve. All subsequent analysis of the soil was carried out in triplicate on this fraction.

Plant preparation.

Plants were harvested from each pot, 5 random ears were separated from the shoot and weighed individually. Great care was taken to avoid cross contamination of any of the materials. Samples were washed in distilled water twice to remove dust and aphid honeydew, air dried, and then dried in at 70^{o}C in a drying oven to preserve until further analysis (Patel et al. 2008). Prior to analysis, plant samples were milled to a powder using a knife mill.

P, K and Cu analysis

The three plant macronutrients are N P K. N was determined as protein as described below and P and K were determined along with Cu as described here. Subsamples of 0.1g of prepared material was weighed out and placed into labelled 50 mL polypropylene tubes. 5 mL of 70 % Primar PlusTrace Metal Grade (Fisher Scientific, Loughborough, UK.) nitric acid was added and the tube swirls to ensure mixing. Tubes were then placed in the digestion block. The digestion block was set to temperature at 40 ^{o}C. After 1 hour the tubes were swirled again and the temperature increased to 50 ^{o}C. The temperature of the digestion block was then increased temperature incrementally (10 ^{o}C per hour) to 90 ^{o}C and heated at 90 ^{o}C for 15 hours or until the tube contents had nearly evaporated. The temperature of the digestion block was then turned down to 50 ^{o}C until the content of the tubes is very nearly dry.

Once cooled the dry tubes and contents were weighed before adding 15 mL of 5 % HNO_3 using an auto pipette, and then re-weighed (Zarcinas et al. 1987). Volume of the digestate was then determined gravimetrically using the measured weights.

Samples were then transferred to15 mL centrifuge tube for determination of P, K and Cu ICP OES (Varian Vista Pro). This analysis was validated by the use of blanks and by the digestion of reference material (CRM 022 for soils and CRM 281 Ryegrass standard in the analysis of plant material). All concentrations are expressed as milligrams per kilogram of dry weight (mg kg^{-1}).

Nitrogen, carbon and crude protein analysis

Nitrogen and carbon levels in plant material were determine by the Dumas dry combustion method using a ThermoFinnegan Flash EA1112 Elemental Analyser. A finely ground 2 mg of sub-sample of material was carefully placed inside tin cup which was then folded around the material. This was then placed in the instrument auto-sampler and the N and C content determined by the analyser using BBOT (2, 5-Bis (5-ter-butyl-benzoxazol-2-yl) thiophene) as a standard and K factor calibration. Crude protein levels were reported by the analyser using a conversion factor of 6.25 X N content.

pH

pH was determined from 5 replicates from each treatment after the pot trails has taken place. Soil pH was determined in 5:2 water:soil suspension following agitation for 15 minutes to produce a soil suspension. pH was then recorded using a pH meter (Thermo Scientific Orion. Ross Ultra, Ross ultra-triode and Ross pH electrodes).

Organic Content

Organic matter content was determined by the loss on ignition following heating to 450 ^{o}C for 10 h.

Statistical Analysis

Statistical analysis was conducted with SPSS (Version 19, 20 & 24). Data sets were analysed for homogeneity of variance with Levene's test. If the data did not pass Levene's then the data were subjected to a Kruskal-Wallis analysis. However all data that conformed to homogeneity of variance was subjected to Analysis of Variance (ANOVA) to test the comparison of means. Differences among ratios were compared non-parametrically with the Kruskal-Wallis test. Spearman's Rank Correlation was used to determine the significance of relationships between the concentrations in soils, leaves and ear samples.

4. Results

4.1 soil

The mean pH of the bulk soil sample was 6.974 (SE ±0.244) and the mean level of organic matter in the soil was 7.24% (SE ±0.099).

Cu

Table 4.1 Table of the Cu content in the amended soils after treatment with $CuSO_4$

Treatment	**Mean Cu mg kg $^{-1}$**	**1 SE**
Control soil	3.207	0.669
25 mg kg $^{-1}$	13.760	2.463
50 mg kg $^{-1}$	20.849	2.835
100 mg kg $^{-1}$	65.667	20.779
200 mg kg $^{-1}$	297.384	30.299

Spiking the soil with Cu resulted in a very large increase in the total Cu concentration in the soil (Table 4.1). A one-way ANOVA confirmed that there was a statistical difference between the Cu concentrations within the treatments ($F_{(4, 20)}$ = 56.111, P = >0.001)

4.2 Plants

Decimal growth stage scoring system is widely used in agriculture as it comprehensively covers cereal development from seed sown to the time of harvest (Barber et al. 2015). Being able to assess observations of defined growth patterns allows for more informed decision making in the management of crops and to optimising inputs of agrochemicals and fertiliser applications to increase yield (Barber et al. 2015; GRDC 2005).

25E 15	200E 18	25C 21	Cont B 16	50D 19
50A 19	Cont D 15	Cont C 15	25B 9	100A 17
100E 17	100D 17	50C 17	50E 13	200D 18
100B 17	200B 17	Cont A 17	200C 16	25A 11
100C 18	Cont E 14	50B 16	200A 19	25D 13

Figure 4.2 A diagram of the distribution of germination rate with in the pot lay-out; approximately 20 seeds were planted in each pot at a depth of approximately 23mm. Upper numbers refer to Cu treatment mg kg $^{-1}$ (dry weight) soil, letter to the replicate of that treatment; lower number denotes the number of seeds germinating.

The mean germination rate was very variable among the treatments (Figure 4.2). Germination rate between the different Cu treatments was compared using a Kruskal-Wallis Test which confirmed that there was no statistical difference between treatment and the rate of germination (H = 8.474, p = 0.076) with the mean rank of 7.50, 8.30, 15.30, 16.40, and 17.50 for treatments Control, 25, 50, 100, 200 mg kg $^{-1}$ respectively.

By day 16 of the pot trial all of the shoots were displaying a 3rd leaf Figure 4.2) of Zadoks Growth stage 10-19 which corresponds to a normal growth pattern (GDRC 2005).

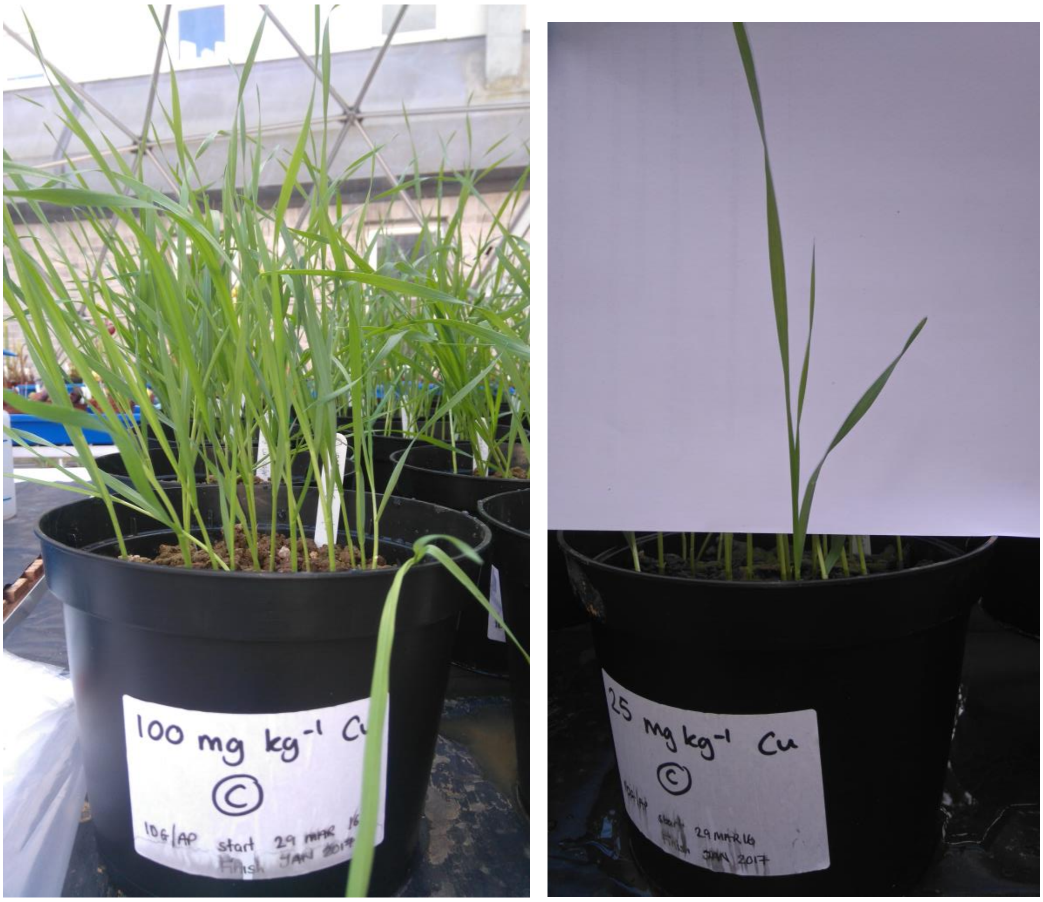

Figure 4.3 Photograph illustrating the development on the 3rd leaf in two pots.

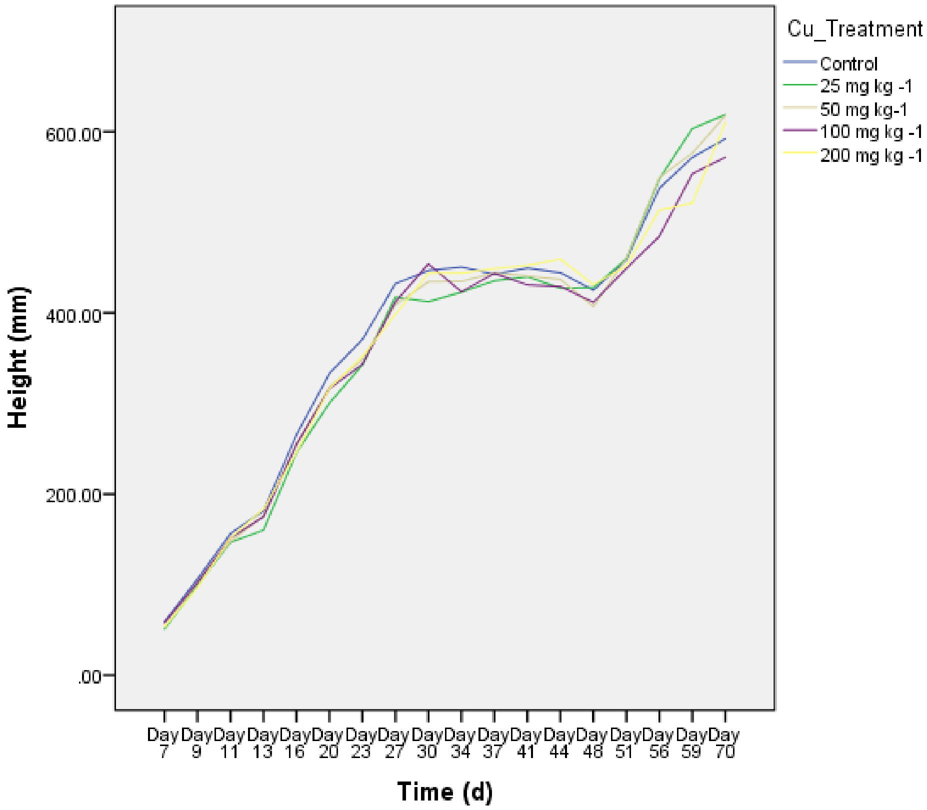

Figure 4.4 The growth of wheat growing in different concentrations of Cu amended soil from planting to Day 70.

Height of the wheat plants increased sharply from germination to day 13 of the experiment (Figure 4.4). The growth rate reduced slightly before rapid growth rates from day 16 to day 27. Growth of the wheat plants then appeared to plateau before increasing after day 44 of the experiment. This coincides with the application of fertiliser.

Measurements from the pot trial were analysed for statistical significance. A two-way analysis of variance was conducted on the influence of two independent variables (Cu treatments on soil, rate of growth over time) on the height of the wheat plants. Cu treatments on the soil were 0, 25, 50, 100 and 200 mg kg $^{-1}$ and the rate of growth was measured regularly between 7 and 70 d of the experiment. All effects were statistically significant, the effect for Cu treatment ($F_{(4, 1259)}$ = 5.487, p = <0.001), and the effect on the rate of time ($F_{(17, 1259)}$ = 1416.636, p = <0.001). The interaction effect between the Cu treatments and time was also significant ($F_{(4, 1259)}$ = 2.021, p = <0.001). Indicating wheat plant growth over time was different among the Cu treatments. However such differences are clearly minor.

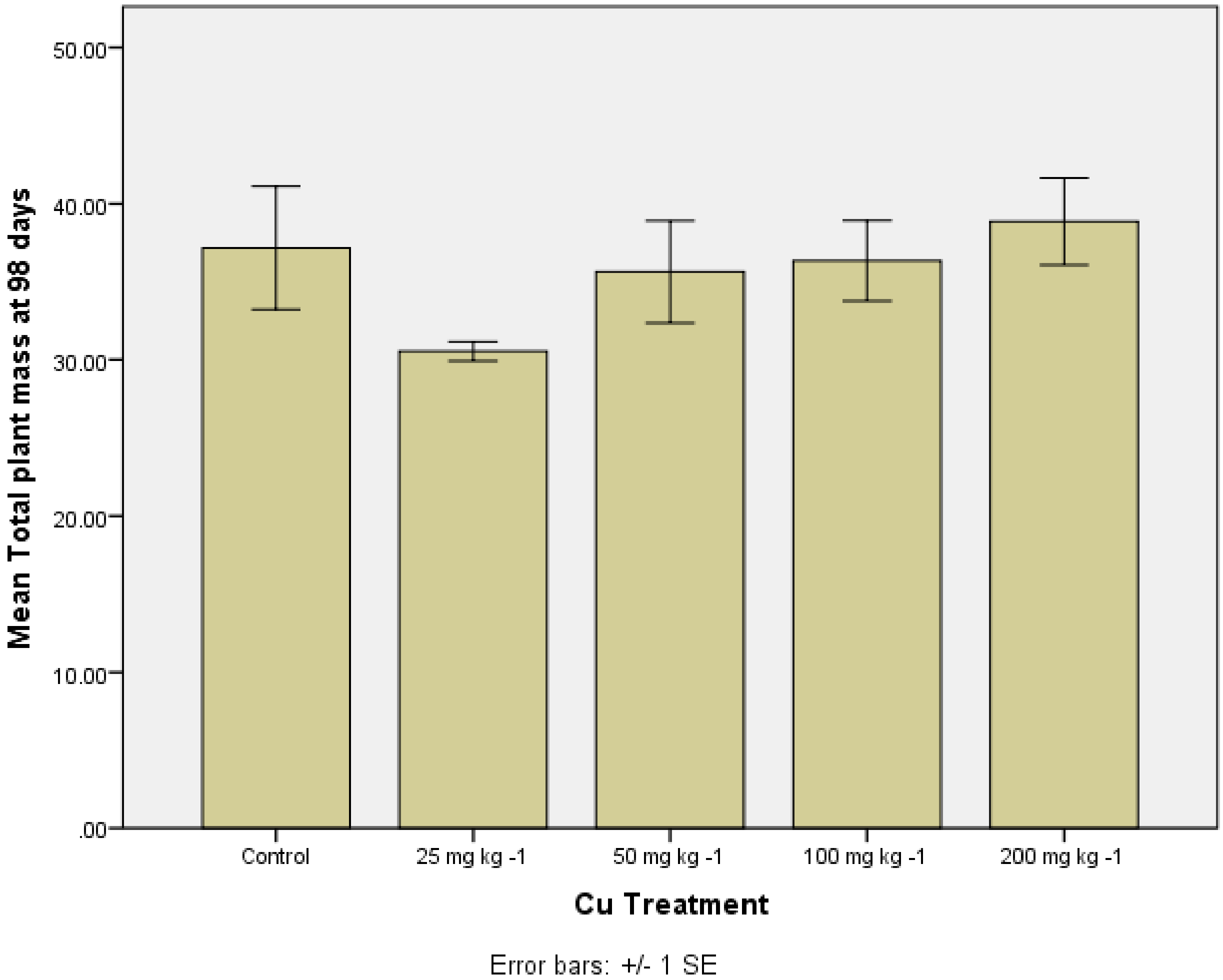

Figure 4.5 The mean total plant mass values from each of the different Cu treatments 98 days from planting (± 1SE).

Differences between total plant mass and Cu treatments showed little difference (Figure 4.5) and the result of a one-way ANOVA analysis showed that there is no significant difference ($F_{(4,20)}$ = 1.190, p = 0.346) indicating that the growth of wheat measured by total plant mass was unaffected by levels of Cu in the soil.

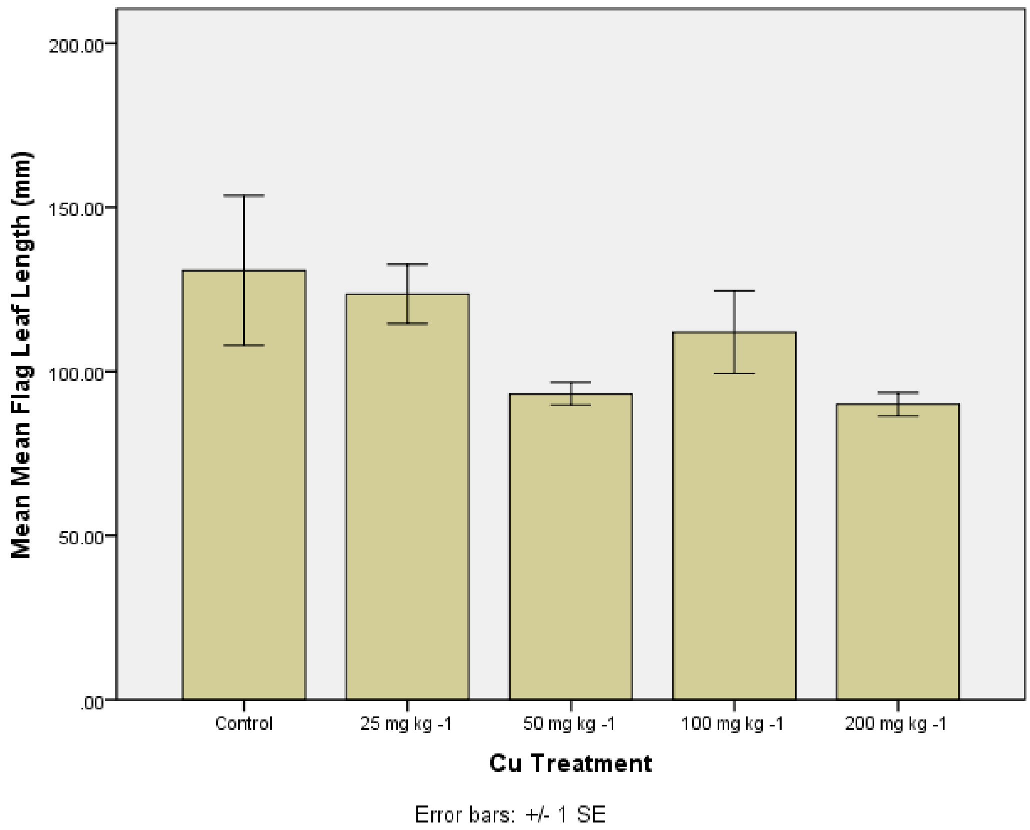

Figure 4.6 The mean flag leaf length at day 98 of wheat plants growing in Cu treated soil (± 1SE).

The mean of the flag leaf length at day 98 showed a decline as Cu treatment increased (Figure 4.6). Flag leaf length between the different Cu treatments was compared using a Levene's test ($F_{(4, 20)} = 3.288$, $p = 0.032$) confirmed that the assumption of homogeneity was not met. So the mean of the flag leaf length was compared non-parametrically using a Kruskal-Wallis Test which confirmed that there was a statistical difference between treatment and flag leaf length ($H = 11.566$, $p = 0.021$) with the mean rank of 17.20, 19.60, 8.10, 13.40, 6.70 for treatments Control, 25, 50, 100, 200 mg kg $^{-1}$ respectively.

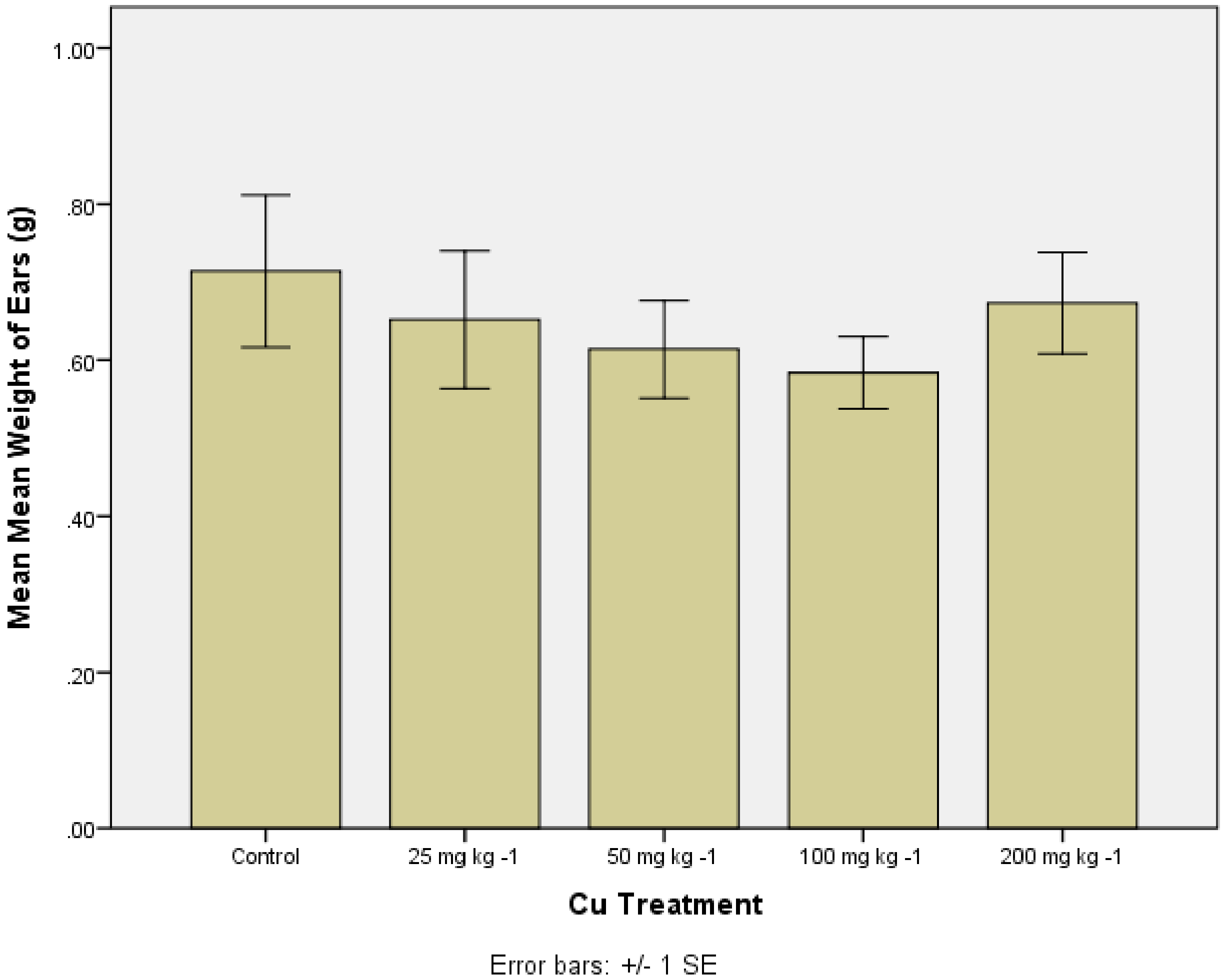

Figure 4.7 The mean weight of wheat ears taken from 5 random plants growing in Cu treated soil (± 1SE).

The mean weight of ears showed a decline as concentrations of Cu increased (Figure 4.7). Differences between weight of wheat ears and Cu treatments were assessed for statistical significance with a one-way ANOVA. The result of this analysis showed that there is no significant difference ($F_{(4, 20)}$ = 0.466, p = 0.760) indicating that the early stages of wheat ear development is unaffected by levels of Cu in the soil.

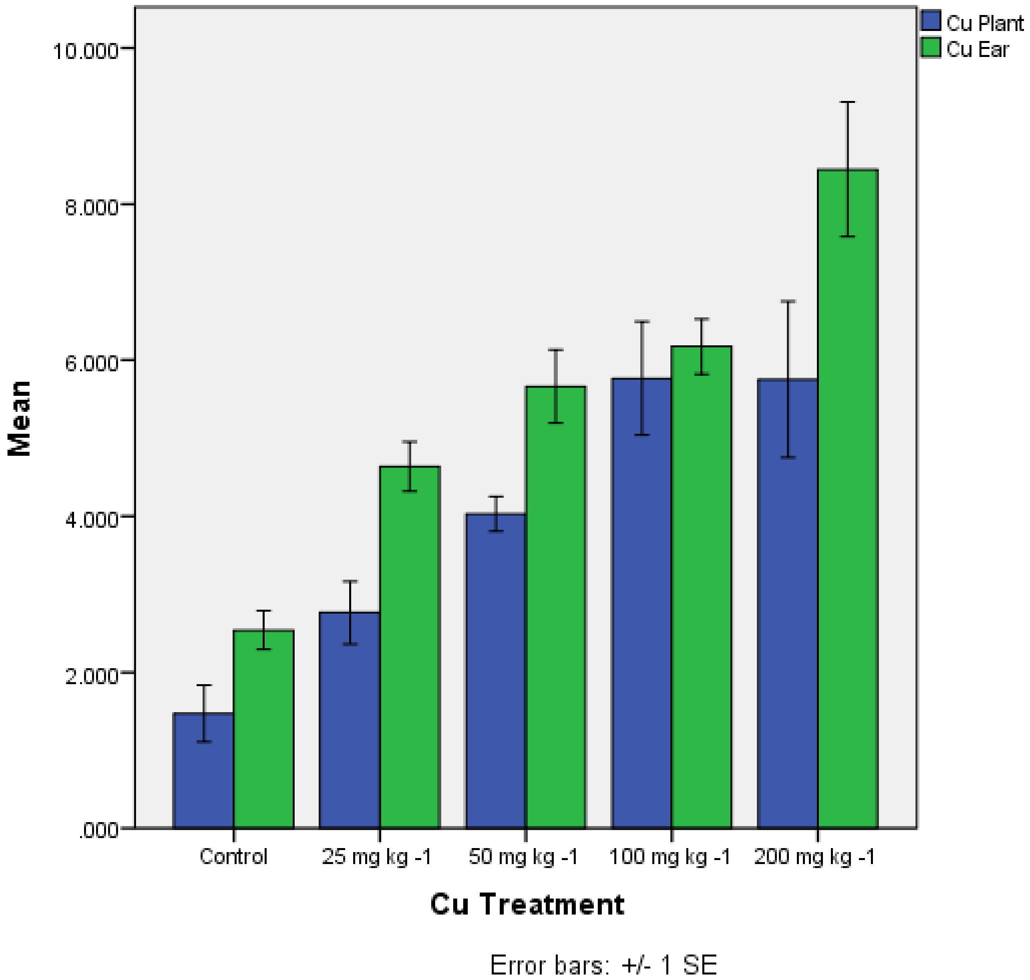

Figure 4.8 The concentration of Cu in shoot and ear tissues of wheat grown in the various Cu treatments (±1SE).

Levels of Cu in the shoot appear to plateau when grown in soil with Cu levels higher than 100 mg kg $^{-1}$ (Figure 4.8), but carried on accumulating in the ear tissues as Cu concentrations in the soil continued to increase.

Differences between Cu concentrations in plant material and ears between the treatments were assessed for statistical significance by a one-way ANOVA. The result of this analysis showed that there was a significant difference for both plant material plant material ($F_{(4, 20)}$ = 9.439, p = >0.001) and ears ($F_{(4, 20)}$ = 18.745, p =>0.001) indicating that Cu did move from the soil through into the plant tissues.

These results were then assessed for correlation with a Spearman's rank correlation analysis which showed significant correlations between soil – shoot (r_s = 0.824, p = <0.001) and shoot – ear (r_s = 0.743, p = >0.001) indicating that there is a significant positive correlation between the transference of Cu between the soil and the different plant tissues.

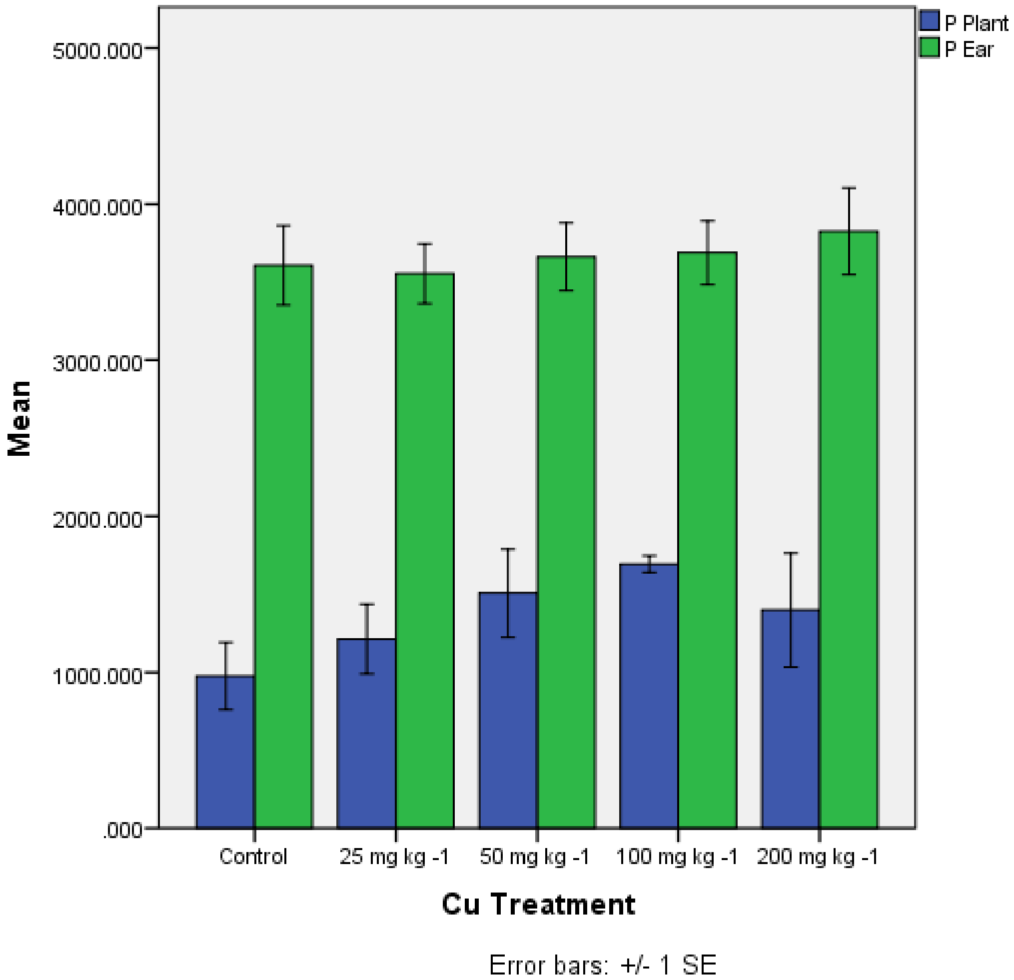

Figure 4.9 The accumulation of phosphorous (mg kg $^{-1}$) through shoot and ear tissues of wheat grown in Cu treated soil (day 98).

There was little difference between the Cu treatments in the mean P concentrations of the ear tissues. However, the mean concentrations of P in the plant tissues increased steadily with the Cu treated soils until 100 mg kg $^{-1}$, mean concentration of P then decreased in the 200 mg kg $^{-1}$ treatment to that below 50 mg kg $^{-1}$. The differences between concentrations of P in the different plants tissues were assessed for statistical significance with a one-way ANOVA. The result of this analysis showed that there is no significant difference between concentrations of P in the different shoot ($F_{(4, 20)}$ = 1.215, p = 0.336) and ears ($F_{(4, 20)}$ = 0.196, p = 0.336) and the Cu soil treatments.

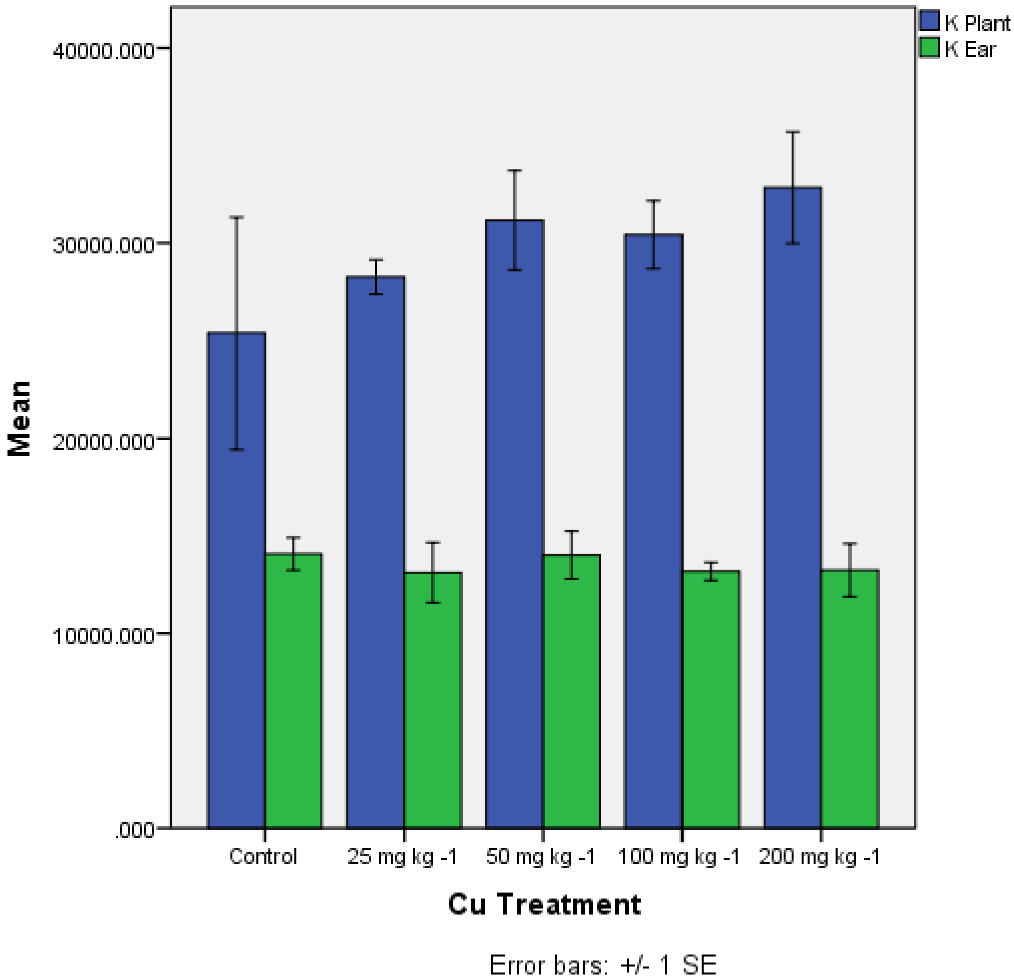

Figure 4.10 The accumulation of potassium (mg kg $^{-1}$) in shoot and ear tissue of wheat grown in Cu treated soil (day 98).

Concentrations of K in the ears showed little variation among the treatments. However K concentrations in the shoot increase slightly with the Cu treatments. The differences between concentrations of K in the different plants tissues were assessed for statistical significance with a one-way ANOVA. The result of this analysis showed that there is no significant difference between concentrations of potassium in the different plants material ($F_{(4, 20)} = 0.772$, $p = 0.556$) and ears ($F_{(4, 20)} = 0.172$, $p = 0.950$) and the Cu soil treatments.

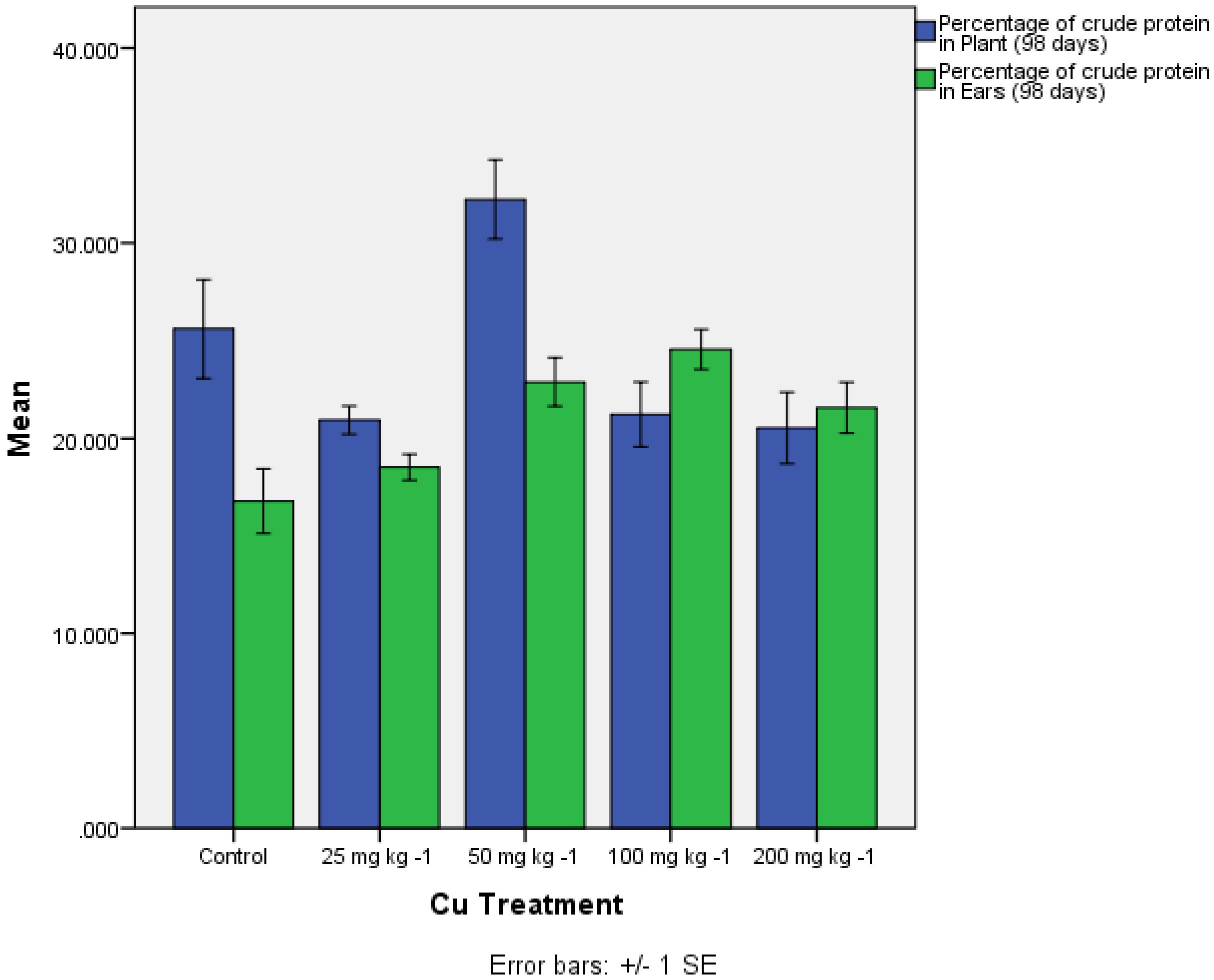

Figure 4.11 The percentage crude protein contained within plant and ear tissues growing in different Cu treated soil (day 98).

Again there was variation between the percentage of crude protein in the plant tissues, there was a peak in at the 50 mg kg $^{-1}$ Cu treatment (Figure 4.11) and then declined as the Cu treatment increased. However, the percentage of crude protein steadily increased to 100 mg kg $^{-1}$ before decreasing in the ear tissue. The differences between the percentage of crude protein contained within plant and ear tissues growing in different Cu treated soil (day 98) were assessed for statistical significance with a one-way ANOVA. The result of this analysis showed that there was significant difference between the percentage of crude protein contained within plant tissues ($F_{(4, 20)}$ = 7.194, p = 0.001) and between the percentage of crude protein contained within ears tissues ($F_{(4, 20)} = 6.340$, $p = 0.002$) and the Cu soil treatments.

4.3 Aphids

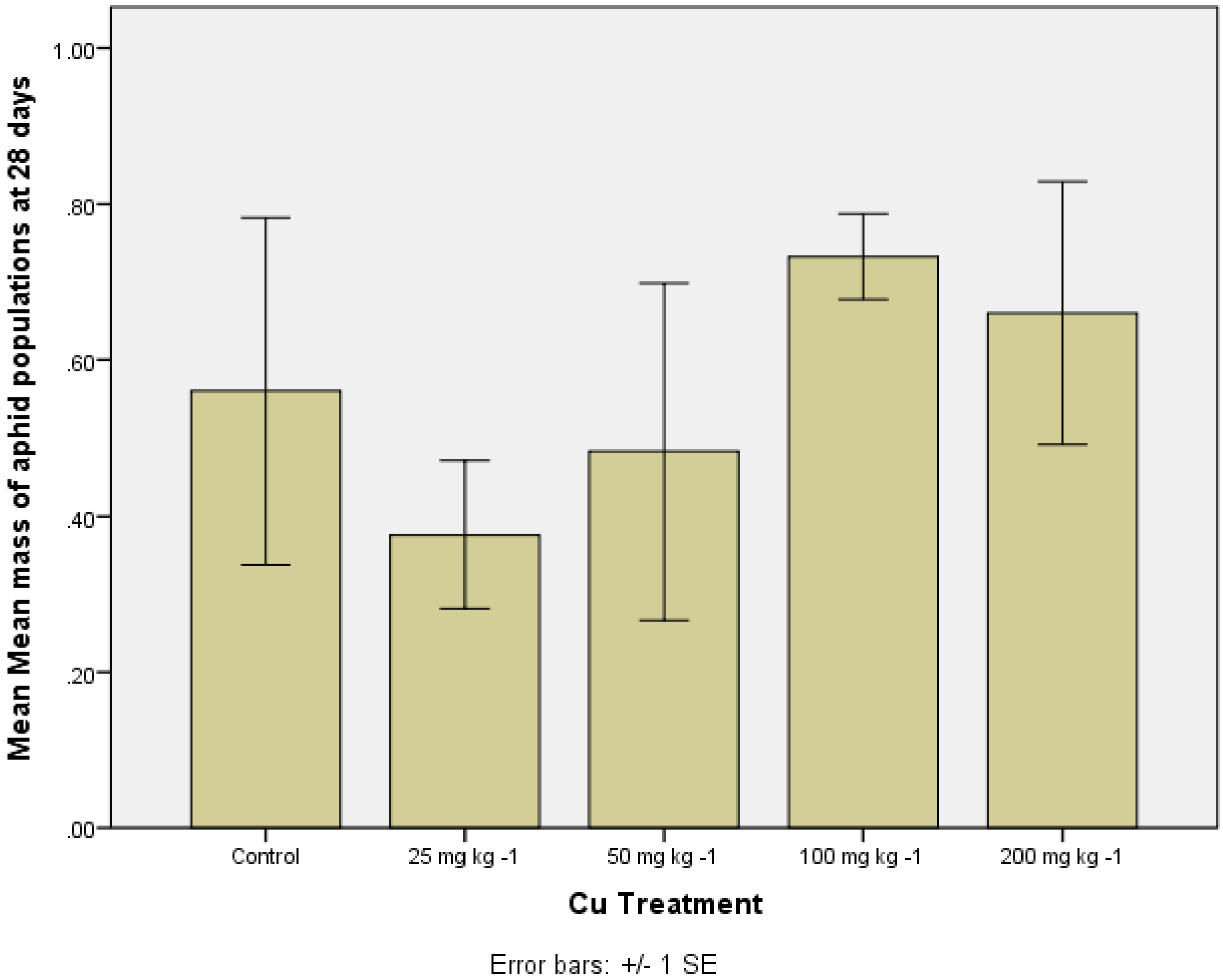

Figure 4.12 The mean populations of aphids (by mass) from five different concentrations of Cu soil treatments (±1SE).

Figure 4.8 shows that there was large difference between aphid population and the Cu treatments which were assessed for statistical significance with a one-way ANOVA. The result of this analysis showed that there is no significant difference between the mass of aphid populations and Cu soil treatments ($F_{(4, 20)}$ = 0.737, p = 0.577).

Throughout the experiment aphids where noted to have a high reproductive rate. Populations of aphids became established within 7 d of being introduced to the pot trails. Live young were observed within 48 h of introduction to the wheat plants.

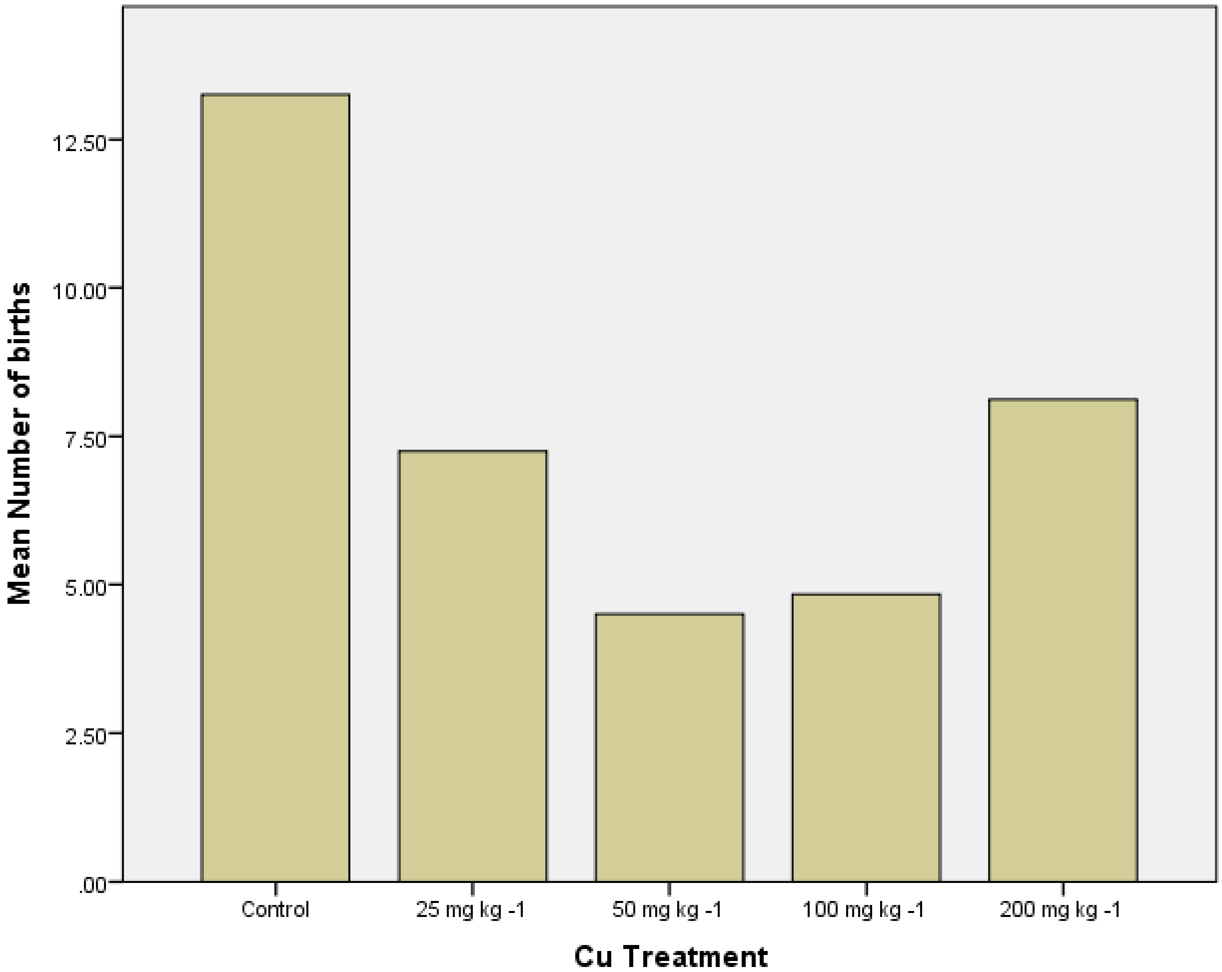

Figure 4.13 Fecundity measured by rate of birth between the five different treatments of Cu soil treatments.

The data show that there is a large drop in births between the 50 and 100 mg kg $^{-1}$ treatments (Figure 4.13). A one-way ANOVA releveled that there was no significant difference ($F_{(4, 20)} = 2.176$, $p = 0.58$) between the number of births and the Cu treatments.

Winged adults were observed in a number of the pot trial by day 93.

Correlations between the plant variables that showed statistical difference were correlated against the total mass of aphid population measurements and measurements of fecundity to establish if any relationships were evident.

Analysis of correlation between aphid population mass and percentage of crude protein in the different plant tissues and the flag leaf length was conducted to investigate these parameters in wheat plants on aphid populations. A Spearman's rank correlation showed that there was no significant relationship between aphid population and the percentage of crude protein in the shoot of the wheat plant (r_s = -0.64, p = 0.776) and no significant relationship between the aphid population and the percentage of crude protein in the ear of the wheat plant (r_s = 0.334, p = 0.139). There was also no significant relationship between the aphid population and flag leaf length (r_s = -0.117, p = 0.604).

Analysis of correlation between fecundity and percentage of crude protein in the different plant tissues and the flag leaf length was conducted to investigate these parameters in wheat plants aphid fecundity. A Spearman's rank correlation showed that there was no significant relationship between fecundity and the percentage of crude protein in the shoot of the wheat plant (r_s = -1.66, p = 0.553) and no significant relationship between fecundity and percentage of crude protein in the ear of the wheat plant (r_s = -0.055, p = 0.844). There was also no significant relationship between fecundity and flag leaf length (r_s = 0.145, p = 0.606).

4.4 Ladybirds

Mortality of the ladybirds was high in the first 48 hours of the experiment with a 32% mortality rate. The frequency of mortality by treatment was compared using a Kruskal-Wallis Test which confirmed that there was no statistical difference between treatment and mortality (H^2 = 4.800, p = 0.308) with the mean rank of 10.50, 13.00, 10.50, 13.00, 18.00 for treatments Control, 25, 50, 100, 200 mg kg $^{-1}$ respectively.

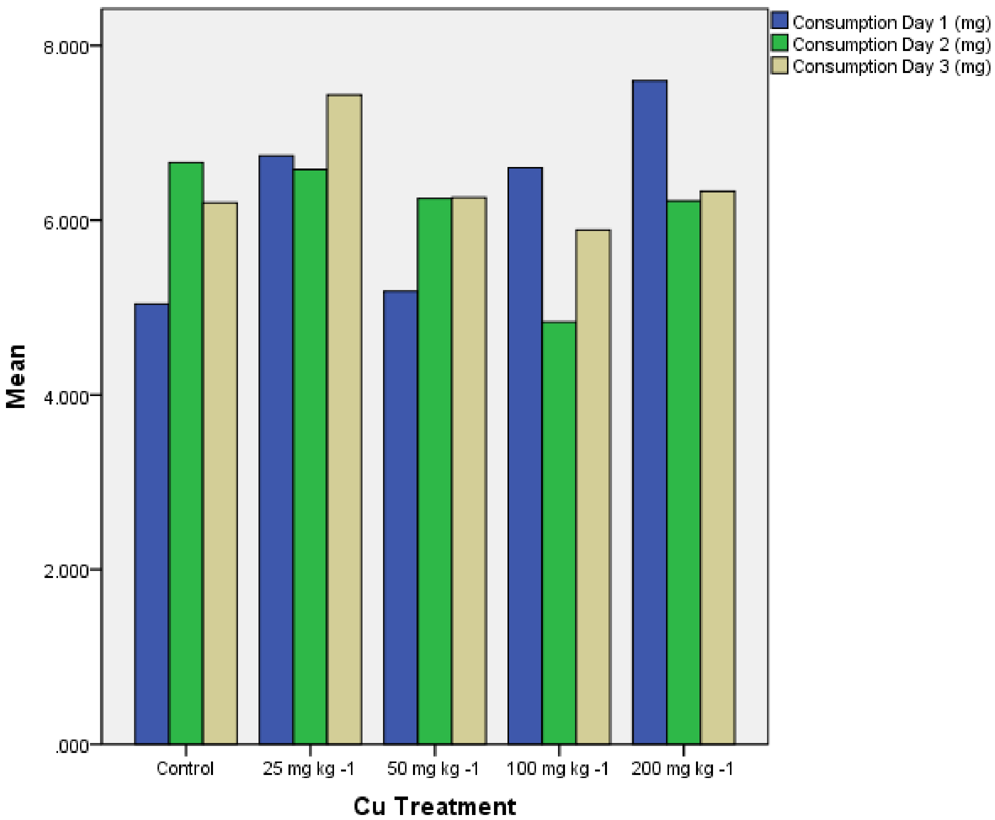

Figure 4.14 The mean consumption weight of aphids harvested from Cu treated plants by ladybirds (mg d^{-1}).

The consumption of aphids by 25 ladybirds over 3 days was investigated during a feeding trial (Figure 4.14). The resulted show that there is no apparent difference in the feeding rate among the treatments. Differences between treatments were assessed for statistical significance by a one-way ANOVA. The result of this analysis showed that there is no significant difference (Day 1 $F_{(4, 20)} = 2.193$, $p = 0.107$; Day 2 $F_{(4,110)} = 0.424$, $p = 0.789$; Day 3 $F_{(4, 12)} = 0.369$, $p = 0.827$) between the consumption rates of aphids affected by Cu treatments in soils.

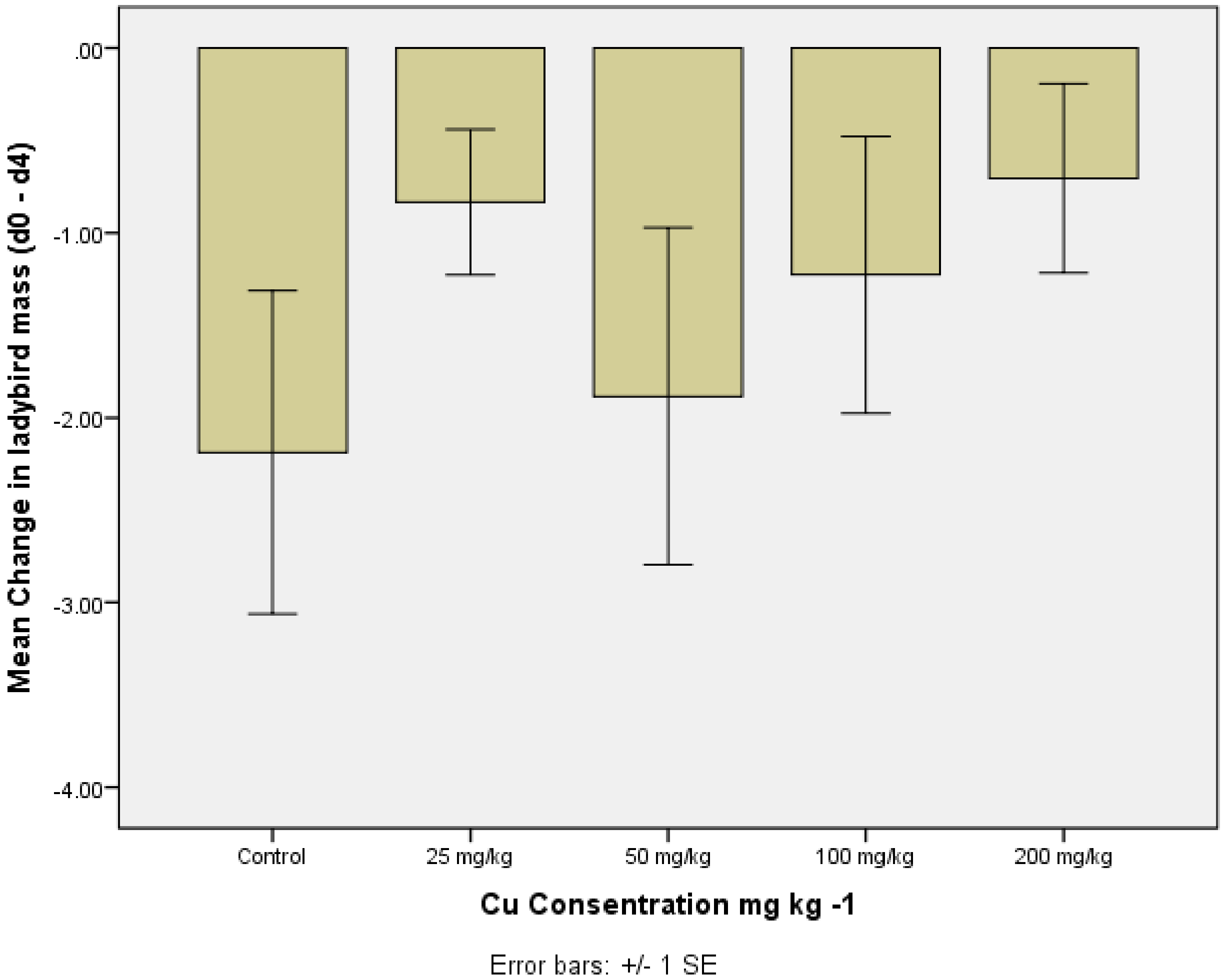

Figure 4.15 Representation of the difference in the mean ladybird mass by Cu treatments from the start and the end of the aphid feeding trails (±1SE).

Though out the feeding trial ladybirds lost mass when feeding form sub-samples of aphids from all of the Cu treatments. The loss of mass differed between Cu treatments with the greatest loss of mass to be from the control treatment.

To determine any statistical difference in the change in mass was compared using a Kruskal-Wallis Test which confirmed that there was no statistical difference between mass loss (H = 2.445, p = 0.654) with the mean rank of 9.60, 14.92, 11.00, 14.00, and 15.63 for treatments Control, 25, 50, 100, 200 mg kg^{-1} respectively.

5. Discussion

5.1 Soils

With an increasing global population there is a high demand for increased food production. Changes to farming practices and farmland becoming more intensively managed, has led to an increased reliance on metal-containing substances in agriculture, especially in the cultivation of cereal crops. In addition, increased anthropogenic pollution emitted through industrial and manufacturing processes is contributing to heavy metal accumulation around the world (Liu et al. 2015). Soil fertility conservation and fertilizer use are vital to crop yield; biologically available nitrogen has more than doubled due to the use of fertilisers in farming (Chaplin et al. 2000). The use of fertilisers and other agricultural chemical is increasing the concentrations of heavy metals within agricultural ecosystems which slowly accumulate in the top soil (Nicholson et al. 2006). The bioavailability of heavy metals is dependent on chemical or biochemical processes such as precipitation-dissolution, adsorption-desorption, complexation-dissociation and oxidation-reduction, all of which are effected by pH and biological processes (He et al. 2005).

The uptake and accessibility of metals can be affected by different climatic and environmental conditions (Stern 1999). The availability of metals is dependent on soil pH and the amounts of organic matter present (Garcia-Salgado et al. 2012). Plants have the ability to increase the pH of soils surrounding the roots to facilitate the uptake of nutrients and heavy metals from the soil solution (DoE 1996) without causing any detrimental effects from high concentrations of H^+ ions to the plants (Menzie 2014). The acidity of the soil implies that the conditions are optimum for the uptake of nutrients and heavy metals since soil pH heavily influences the bioavailability of these molecules (Stern 1999; Peralta-Videa et al. 2009).

Most plants prefer slightly acid soils as it provides nutrient rich conditions required for plant growth such as nitrogen, phosphorus, potassium and sulphur that all become readily bioavailable in acid soils (Ovecka & Takac 2014: Bohn et al. 2001). The mean pH of soil used in this experiment was 6.974 (SE ±0.244) slightly higher than the ideal pH for agricultural soil which is pH 6.5 (O'Hare 1988). Since the more acidic the soil solute the more easily cations are removed from clay and organic matter particles, the more neutral pH would imply that the plants grown in this soil did not have the maximum availability of Cu as this would have been bound to the exchange site sites on the surface of the organic matter particles.

Generally, concentrations of heavy metals available to plants increase consistently with that contained in the soil (Brunetti et al. 2012) so the increased concentrations of Cu within the treated soils would have been expected to be taken up and potentially influenced the growth and development of the plant consistently. In this experiment it was shown that the levels of Cu in the soils did affect growth of the wheat plants over time but this did not affect the total mass of the plant only the length of the flag leaf and the decline in ear weights as the Cu concentrations increased.

The movement and retention of soil solute is governed by the organic matter content of the soils and the surrounding geology (DoE 1996; Garcia-Salgado et al. 2012). There has been a decrease in the organic matter content in soils over the last 50 years due to changes in farming practices and long-term mono-cropping. Nutrient shortfalls have been met with the increased use of mineral based fertilisers to maintain productivity and yields (Loveland and Webb 2003). If the organic content of the soil decreases below a certain threshold then the productive capacity of the soil becomes compromised (Loveland & Webb 2003). The soil used in this experiment had a loss on ignition of 7.24% (SE ±0.099) with high clay content, this in inline for clay soils in the UK which has the soil organic matter content in the range of 1.2-9.9% with a mean organic matter content of 3.38% (Loveland & Webb 2003). The high clay content facilitates the retention of water within the soil medium, combined this with neutral soil conditions provided poor conditions for ionic exchanges among the soil particles (FSA 2012). This makes nutrients and heavy metals in the soil less bioavailable, as they are regulated by the adsorption and desorption characteristics of the soil, dependant on a higher pH (Ran et al. 2016).

Cu

Cu, Ni and Zn are not included in European Legislation as food contaminants as they are considered to be essential for plant development (Brunetti et al. 2012). The maximum threshold value for Cu that can be tolerated for agricultural crops, fruits and vegetables is 50 mg kg^{-1} (Langenkamp et al. 2001). Distribution of copper across Europe is the most complete data set but far from complete or standardised between the member states as levels of Cu are not regulated (Langenkamp et al. 2001; Zaccone et al. 2010). In the UK, the range of Cu levels in rural and urban soils have considerable ranges of 2.27 – 96.7 mg kg^{-1} and 8.27 – 181 mg kg^{-1} respectively (EA 2007). The mean Cu content for rural soils is 20.64 mg kg^{-1} for rural soils and 42.50 mg kg^{-1} for urban soils. The soil treatments in this experiment were within the acceptable ranges for agriculture except from the 200 mg kg^{-1} which was above both the range of rural and urban soils.

Long term low-level mixed metal exposure is complex and interactions are difficult to quantify as they are heavily dependent on soil characteristics, pH, and organic matter and texture (Ran et al. 2016). Standards are based on single metal toxicity a more comprehensive understanding of mixture toxicity would be desirable when assessing associated risks ad setting regulation standards (Son et al. 2016). In the UK it is considered good practice to regulate and monitor agricultural applications of fertilisers, including sewage sludge, herbicides, pesticides and any other chemicals applied to the soil (Nicholson et al. 2006) however this is not compulsory.

With over a third of the world's soils are at risk from degradation (Rojas et al. 2016) and many countries having a high prevalence of heavy metal contamination as well as environmentally damaging technologies (Tang et al. 2015), there is need for soils to be sustainably managed as they are required to supply food for growing populations (Johaus et al. 2016). Both ecosystem services and crop production are underpinned by soil characteristics, and changes to soil characteristics can affect the productivity of the soil and therefore impact both food production and ecosystem services.

5.2 Plants

Bottom-up processes are governed by plant quality either by changing the nutritional status of the host plant or by increasing levels of toxicity (Butler & Trumble 2008). Bukovinszky's (et al. 2008) investigations suggested that variations in plant quality have an effect on subsequent trophic levels, since herbivorous insects obtain all their nutrients from host plants. The quality of the host plant has a direct effect on the performance and fecundity of these insect populations (Awmack & Leather 2002).

Plants suffer reduced energy production from compromised growing conditions and divert energy towards plant protective mechanisms, such as the redistribution of essential resources and the synthesis of toxic or anti-nutritive compounds (Menéndez et al. 2011). The quality of a host plant is variable; there can be predictable changes in the quality to due seasonal or developmental change or unpredictable changes caused by environmental stress (Awmack & Leather 2002). As previously described, heavy metals can enter agro-ecosystems through a number of different pathways, atmospheric deposition, fertilisers (organic and inorganic), industrial by-products and other agricultural applications, thus Cu, Cd, and Cr are more bio available for uptake by wheat plants (Zaccone et al. 2010).

Accumulation of heavy metals is dependent on the genotype of the wheat variety. Brunetti (et al. 2012) suggests that wheat grows normally under heavy metal stress conditions without and reduction in plant biomass, accumulating metals in the roots. It is understood that Cu soil concentrations becomes phytotoxic to wheat plants above 120 mg kg $^{-1}$ (Dimkpa et al. 2012), in this experiment that level was only reached 200 mg kg $^{-1}$ treatment when the Cu concentrations in the soil reached 297.384 mg kg $^{-1}$. Wheat is known to exclude Cu from root to shoot translocation when growing in Cu-rich soils (Brunetti et al. 2012; Lanaras et al. 1993) accumulation Cu in the roots to almost twice that of the levels ion the soils, this suggests an avoidance strategy to prevent the accumulation of heavy metals in plant tissues. In this experiment the germination and growth rate did not appear to be affected by the Cu treatments within the soil, but Cu was taken up into the plant tissues.

Plant mechanisms to retain or distribute heavy metals are species specific and dependent on environmental conditions (Brunetti et al. 2012). The results from this present investigation showed that there was a significant correlation in the transference of Cu from the plant material to the ear. Studies by Wang et al. (2013) also demonstrated a transference of Cu in wheat plants to the developing ears, and stressed that increased concentrations of Cu in the plant tissues was also correlated to the accumulations and transference of other molecules namely, sulphur and iron. However the correlations between soil and plant concentrations of Cu are inconsistent (Green et al. 2006; Guan et al. 2011; Wang et al. 2013). Indicating that both genetic and environmental factors affect the biochemical functioning within wheat plants (Jiang et al. 2014) and there is variance through the varieties of wheat and the conditions in which they are grown.

The wheat plants in this study were observed to meet defined growth stages within the recommended time frames in accordance with the Zadoks et al. (1974) scale. Three leaf stage is a critical growth stage in the development of wheat as it marks the transition from autotrophic to heterotrophic growth (Jiang et al. 2016) and indicates that the seedlings were unaffected by the elevated levels of Cu in the treated soils.

Whilst the rate of growth was not significantly different between the treatments (Figure 4.4.), the application of NPK fertiliser (Day 41) is clearly illustrated among all the treatments this is also confirmation that the wheat plants were growing unaffected by the levels of Cu in the soil. These results reflect findings from Dimkpa et al. (2012) that stated that Cu did not affect the length of wheat plants when grown in hydroponic sand systems to investigate the effects of Cu and Zn on wheat growth. There was also no significant difference in the total above ground plant mass after 98 day of growth in the cu soil treatments. This is in line with current research that suggests that wheat is unaffected by Cu levels in the soil as Cu is excluded or held within the root (Brunetti et al. 2012). However root growth and development is affected by the Cu levels in the soils, reducing mass and modifying the root structure (Dimkpa et al. 2012). In the present study root growth was not investigated and so was not included in the calculations in total plant mass.

Measurements of the flag leaf on day 98 of the experiment showed that there was a significant difference between flag leaf length in plants growing in difference concentrations of Cu treated soils. This is significant as the flag leaf provides the majority of the photosynthetic energy required for grain filling as the ear of the plant matures (GRDC 2005). The length of the flag leaf is a key indicator to grain yield, as the larger the area of the flag leaf the greater the photosynthetic capabilities so the greater production. This change in flag leaf length suggests that plant is under some stress due to the Cu treatments and had fewer resources to put into flag leaf growth.

The ears of the wheat plants taken from each of the pot trials and subsequently weighed did not show any statistical difference between treatments indicating that the concentration of Cu in soils does not immediately affect the growth and development of wheat grains. This contradicts the previous result of the flag leave length corresponding to grain yield and hence ear development (Al-Tahir 2014). The present study was conducted over 98 d and ears had yet to fully mature and so it is difficult to conclude grain would not been affected by the levels of Cu in the soil.

There have been a number of studies into the effects of accumulation of various heavy metals and the relationship between the heavy metals and other nutrient elements in a soil-wheat system (Wang et al. 2013). In the present study it is clear that as Cu increases in the soil, it also increases in the plant and ear of the wheat. However there was no significant difference in the uptake or phosphorous or potassium or any correlation between soil-plant-ear transference of these nutrients.

By contrast there were statistical differences in the percentage of nitrogen and crude protein within the plant tissues. Results showed that there was an increase in both nitrogen and crude protein in the ear tissue of the wheat plant between Cu treatments 0 - 100 mg kg $^{-1}$. Since there was no statistical difference in the ear mass it cannot be a concentration response but rather a metabolic response. A study by Jiang et al. (2014) found that the production of amino acids (i.e., proline, phenylalanine and histidine) were greatly influenced by environmental factors, and wheat grain is known to have a high variation in the amino acid composition (Panda et al. 2003; Spychay-Fabisiak et al. 2014). Methionine, aspartic acid, glutamic acid and serine are relatively stable in quantities within various wheat varieties whereas lysine, isoleucine, histidine, alanine and leucine have the highest fluctuation rates in wheat grains (Spychay-Fabisiak et al. 2014).

Proline has been found to accumulate over time with increased concentrations of heavy metals (Panda et al. 2003). As the percentage of crude protein has increased in both the plant and the ear with the levels of Cu in the soil treatments, it can be deduced that there has been an accumulation of proline and other amino acids as a part of a defence response to the Increased Cu in the plant tissues.

The variety determines the grain yield and quality (Spychay-Fabisiak et al. 2014). The higher the protein content the higher the grain quality, ideally the total protein in fully developed grain of Tybalt should be 12.8% (HGCA 2013), although the ears of the wheat were not fully developed the levels of crude protein was above this. Development of wheat varieties that are tolerant of heavy metal contaminants and have increased pest resistance may provide environmental solutions (Alybayeva et al. 2014; Wanlei et al. 2009) to heavy metal contamination and the improvement of protein content within wheat varieties. It is thought that genetically engineered crop plants could be created to increase the levels of proteinase inhibitors to provide increased pest resistance (walker et al. 1998) and simultaneously increasing the levels of crude protein in wheat grain.

5.3 Aphids

Heavy metals pose a threat to ecosystem integrity. Whilst it has been recognised for several decades that the transfer of heavy metals through food chains could alter interactions between secondary and tertiary consumers via secondary poisoning (Awmack & Leather 2002; Bukovinszky et al. 2008; Gorur et al. 2007; Mendéndez et al. 2013), there has been no evident work to investigate whether heavy metal stress of the primary producer affects subsequent relationships in the food chain in the absence of metal transfer.

Cu intake is regulated by aphids; any excessive amount of Cu consumed is excreted in honeydew and therefore not accumulated in body tissues (Crawford et al. 1995). This isolates aphids from the toxic effects of Cu (Crawford et al. 1995), so any changes to plant-herbivore or herbivore-predator relationships will be due to Cu causing a stress response to the host plant. Therefore the populations and condition of each trophic level will be affected indirectly by the environmental conditions (Harmon et al. 2009), in this instance, levels of soil contamination.

Aphids have to consume large quantities of food to acquire enough protein for maintenance and development (Dixon 1973). The use of nitrogen based fertiliser increases plant quality and consequently the nutritional suitability for herbivorous insects enabling the establishment large populations (Lohaus et al. 2013). Plants that are already under stress suffer a greater impact due to direct feeding damaged due to lower soluble carbohydrates in the phloem (AHDB 2014). Different aphid species respond differently to the nutritional quality of plants, but it is assumed that plant quality will affect the fitness of all aphid species (Lohaus et al. 2013).

Aphids are most prolific when plants are becoming established at which point phloem is high in amino acids and low in deterrent compounds (Awmack & Leather 2002). Proteinase inhibitors are present in much of the plant tissues; they have numerous functions including defence mechanisms. When ingested by pest species proteinase inhibitors bind to proteolytic enzymes in the gut and interfere with the digestive process of the pest (Walker et al. 1998). For more mature plants any compromise to growing strategy allows for the aphids to take advantage to maximise reproduction and so increase population sizes. When populations of aphids reach maximum densities or host plants cannot supply adequate nutrition to maintain the populations, this triggers the morphing of winged aphids to be produced allowing for large scale migrations (Xin et al. 2014). This was observed in some of the pot trials around Days 93 – 95 when there was a sudden increase in the observed number of winged adults.

Results for the present study found that there was no significant difference in the mass of aphid population mass and the levels of Cu in the soil, aphids appeared to be unaffected by all treatments. Correlations analysis showed that there were no significant relationships between aphid populations and flag leaf length, crude protein in the wheat shoot or the wheat ears which had shown to be significant in relation to the Cu treatments in the soil. Fecundity also showed that between the Cu treated soil and no significant relationships between flag leaf length, crude protein in the wheat shoot or the wheat ears. These results cannot provide conclusive evidence of fecundity on the different plant parameters due to the short time they were on the plants.

Aphids become pests at any point of the development of crops; in the UK wheat is sown in early spring as aphids beginning to emerge from over wintering. Cereal crop development can be divided into three distinct phases: slow expansion phase (Growth stage (GS) GS13 – GS30) when nitrogen utilisation is low, rapid expansion phase (GS30 – GS60) when nitrogen usage is at its peak and the senescence phase as the wheat's resources are redistributed to the grain filling (GS60 – Harvest) (GDRC 2005). Wheat flowering is the peak of aphid colonisation, populations then decrease as the wheat ripens and the resource availability declines (Lohaus et al. 2013) as the nitrogen and phosphorus content in the plant decreases with age (Amwack & Leather 2002). During this experiment it was observed that the aphid populations quickly became established, aphids were introduced to the wheat plants during the rapid expansion phase of wheat development ensuring that there was optimum amounts of nitrogen in the phloem to support an aphid population. Live young were observed on the wheat leaves within 48 hours of introduction suggesting that conditions of the netted pot trails were adequate for colonisation.

Sitobion avenea, the grain aphid, can utilise a variety of host plants (Xin et al. 2014) they feed on the leaves and stem of a plant before moving the ears where they feed on the phloem being supplied to the grains (AHDB 2014). In this experiment results indicate that there is no statistical difference between fresh mass of aphid population and Cu soil treatment, total plant mass at 98 days or the mean ear weight. Thus, whilst the plants show some indications of experiencing stress, the system is not under sufficient stress to cause a significant response in the herbivore.

Increased levels of soil nitrogen increase the performance of both the wheat and aphid populations. Between 2010 - 2011 57.5 Mt of nitrogen fertilisers were applied to cereal crops globally with wheat production the primary recipient accounting for 18.1% (Heffer 2013). Nitrogen deficiency is often to blame for reduced yield in wheat production (Liu et al. 2014) and so fertilisers are often applied to crops to increase the yield, inadvertently improving the conditions for aphid populations (Awmack & Leather 2002).

Research into farming practices is conflicting; Bengtsson (et al. 2005) indicated that there was no significant difference in the abundance of pest species such as aphids with the use of organic or conventional farming methods. Whereas Lohaus (et al. 2013) stated *S. avenea* are more prolific in organically managed fields than in conventionally managed fields. The experiment revealed that species composition of aphids were different on organically managed fields where 96% of aphids where *S. avenae,* whereas in conventionally managed fields *Metoplohium dirhodum* and *Rhopalosiphum padi* were equally dominant, making up 79% of all aphids.

Conventional farms may even increase the abundance of aphids due to the use of pesticides reducing the number predatory insects, while the lack of pesticide usage in organic farming methods are also advantageous to pest species such as aphids (Bengtsson et al. 2005). In the present study, *S. avenae* were used due to their association with wheat and their prevalence in organically managed farms as, organic farming methods have the highest used of agricultural applications with the greatest Cu content namely sewage sludge application, application of Bordeaux mixture and farm yard manures.

Aphids are also vectors for a number of diseases that can also cause a loss in yield and can cause indirect damage as the honeydew excreted by aphids provides a medium for moulds and food source for other pest species (AHDB 2014). Many cereal crops have been developed with disease and mould resistance to avoid pest damage (HGCA. 2013).

5.4 Ladybirds

Ladybirds are heterogeneous feeders; it is recognised that both prey availability and prey type play a major role in the survival and development of ladybirds. Limited or poor quality prey results in reduced developmental larvae rate and the resulting adults being smaller in size (Omkar 2014). It is understood that changes to the nutritional quality and composition of plants can also affect both adults and ladybird larvae through tri-trophic food chains (Walker et al. 1998), even though the transfer of heavy metals declines through each of the trophic levels (Zhuang et al. 2009).

In the present study adult ladybirds were used in the feeding trail. Investigations by Green & Walmsley (2013) suggested that Cu accumulation in ladybirds did not vary significantly indicating that Cu intake is heavily controlled in the species. Consequently, despite increased levels of Cu in plants, transfer to the ladybirds would have been limited by control in both aphids and the ladybirds themselves. So, any changes in the herbivore-predator relationships will be due to Cu causing a stress response to the host plant and not the accumulation of the heavy metal itself.

The mortality rate 32% of ladybirds was highest at the beginning of the experiment during day 1. This is not an unusual finding for lab based experiments with ladybirds and was most likely due to the weaker individuals succumbing to the stress of the feeding trial conditions or the individuals reaching the end of their natural lives (Green and Walmsley 2013). Mortality after the initial period was 0% suggesting that conditions of captivity were adequate. There was no significant difference between the amounts of aphid consumed from the Cu treatments indicating that increasing levels of Cu in the soil does not affect the predation rate of ladybirds, at least not in a no-choice feeding trial. However, the loss in mass of the ladybirds does imply that the aphid only diet was sub-optimal. This may have been due to the aphids having been frozen ideally live aphids would have been preferable.

It is generally understood that aphids have a low nutritional value to polyphagous predators such as ladybirds (Bilde & Toft 1994). The diet of frozen aphids did not appear to be unpalatable as consumption did not decline over time. The present study shows that there were no significant differences between the rates of consumption of the aphids. However the weights of the adults before and after the feeding trial did emphasize an array of sizes between the individual subjects. This is not unusual in arthropod populations (Merrington et al. 2001) and could be a result of age and or sex of the individual ladybirds. As previously stated the size of the ladybird does affect the amount of food that needs to be ingested to maintain survival, so some ladybirds may have had bigger appetites than others.

Although the present study does not provide evidence for the levels of Cu in soils affecting the higher trophic levels it does not rule out the biological significance of elevated levels of heavy metals in soils have an concomitant effect on ecosystem functioning. The inadvertent applications of heavy metal on agricultural soils may be impacting unseen trophic interactions. Environmental disturbances affect species abundance not just through the evolutionary response of the species but also how that species interacts with other species in the food web – the ecological response. Both the evolutionary and ecological response can operate on different time scales (Harmon et al. 2009). Therefore environmental disturbance, such as soil contamination may not directly interact with the ecological and evolutionary process so an observable or measurable difference may yet be seen.

5.5 Ecosystem services.

Biodiversity and ecosystems are under pressure (EC 2014). Habitat fragmentation is affecting biotic interactions and changing the community structure. The more specialised the species the more vulnerable they are to habitat degradation (Tscharntke et al. 2002). It is difficult to assign an economic value to ecosystem services provided by insects, as such conservation efforts and research remains a low priority (Losey & Vaughan 2006). Nonmarket value of agri-ecosystems biodiversity or ecosystem services are not measured on the same scale as agriculture so are deemed less important (Smith et al. 2009). Much of central Europe is dominated by agricultural land, with little space being set aside for conservation (Tscharntke et al. 2002). Over 75% of the UK is categorised as agricultural land with 40% being arable fields and grasslands (RSPB 2016). This accounts for a huge area at risk from degradation and loss of biodiversity among a large range of species.

The present study has not provided clear evidence that the levels of Cu in the soil are causing an effect of agroecosystems. However, the findings do imply that there are some interactions that would need to be investigated further. Manipulation of the environment such as agriculture does add additional stressors to an ecosystem. Intensive management of agricultural land has the largest negative impact on nature across the UK (RSPB 2016). Success of a species is it's response to change through adaptations or tolerances within physiological behaviours and life histories over multiple generations, as well as how that species interacts with other species in the same ecosystem (Harmon et al. 2009). Out of 1118 farmland species 137 (12%) feature on the Red List and farmland bird indicators show a decline of 54% since 1970 (RSPB 2016).

UK environmental legislation is underpinned by EU legislation the vote to leave the EU will mean that these legislations will need to be made into UK law to be enforced. Environmental stewardships can be achieved by increasing area covered by semi-natural habitats increasing food and shelter available to wildlife species and reduction of pesticide and fertiliser use (RSPB 2016). Environmental stewardships are currently co-funded under the EU Common agricultural policy (CAP) the UK vote to leave the EU puts these schemes at risk (RSPB 2016).

Biological control

There is an increased awareness of the benefits of using natural predators for pest control (Weibull et al. 2003). It has been suggested that the economic value of pest control, of herbivorous insects by natural predators is around 4.9 billion US$ per year, but these beneficial insects are under increasing threat from habitat destruction, invasion of foreign species and the over use of agricultural chemicals (Losey & Vaughan 2006). For optimum biological control, ladybirds require high quality prey to ensure larvae have high consumption and growth rates, fast development and low mortality. The emerging adults will be larger in size and so will also consume more aphid prey, produce more eggs and therefore produce more larvae (Omkar 2014).

Spatial arrangements of habitat fragments are an important factor in enhancing naturally occurring biological control populations (Tscharntke et al. 2002). There has been a steady decline in beneficial insects which has been associated with overall decline in biodiversity and habitat degradation within agri-ecosystems (Losey & Vaughan 2006). Species richness increases with heterogenous landscapes as they provide a variety of habitats and plant structures (Weibull et al. 2003). Predator species depend on resource found in non-crop habitat, such as alterative prey, pollen and nectar and over wintering habitats (Tscharntke et al. 2002). There are often multiple species performing similar functions so declines in one species may be compensated for by another with no loss in ecosystem functionality (Losey & Vaughan 2006).

Climate Change

Climate change will impact the diversity and structure of soil communities, leading to changes in soil processes, movement of water, gases and on the decomposition or organic matter (Morecroft and Speakman 2015) which will all affect the growth of plants and the production of crops. The breakdown of organic matter is known to be faster in warmer climates (Loveland & Webb 2003) increasing the degradation of soils through soil erosion (Brady & Weil 2008). This will impact the accumulation and retention of heavy metals in the soils. Agricultural systems are affected by short term weather systems or impacts of weather events such as flooding could provide the mechanism for the release of organic matter and bound heavy metals into the wider environment and water systems

Even though wheat can be grown in a variety of climatic conditions, changes in climate will have a dramatic impact on yield (Hawkins et al.

2013). Increasing temperatures increased with climate change may create shift the primary production areas for growing wheat (Shifeaw et al. 2013). It is estimated that since 1980s total global yields have already decreased by 5.5% (Lobell et al. 2011; Shifeaw et al. 2013). To insure global food security there needs to be a development of new wheat varieties and management practices to maintain and increase yields to kep up with global demand (Shifeaw et al. 2013),

Prolonged dry spells change soil dynamics; it slows down the leeching of soluble nutrients and the disassociation of molecules into soil solutes and results in the reduction of many microorganisms (Sanchez et al. 2003).

Response to environmental change is becoming increasingly important to biodiversity, ecosystem and agricultural production (Pocock et al. 2012). Changes in temperature can have profound effects on the biochemistry and physiology of invertebrate species (Porcelli et al. 2016). Changes in climate have resulted in changes of species distribution and phenology especially herbivorous insect species, especially in species that have short generation times (Davies et al. 2006; Morecroft & Speakman 2015).

6.0 Conclusion

This study is of economic and ecological importance in both assessing the current status of Cu levels in soil and safeguarding future food production and land management practices.

A single critical threshold value for Cu content in agricultural soils cannot be supported from the evidence available in the study. Levels of Cu in treated soils did not affect germination of the seeds but there was an effect on the rate of growth between the treatments which did not affect the development of the wheat plant. Plant mass or the mass of the developing ears was unaffected by the Cu treatments. However the length of the flag leaf was affected by the levels of Cu in the soil, which may account for the effect on the protein composition in the developing ears and may impact the final grain yield with economic significance.

In the investigation the stress endured by the plant did not affect the mass of the aphid populations and there were no correlation effects between soil treatments, total plant mass, flag leaf length or the average ear weight and the mass of the aphid population. However, aphids were only present on the wheat plants for 28 d, there may be a difference response on aphid populations over an increased time as there were a high number of winged adults present at 93 d indicating that that host plant suitability was declining.

It is the current understanding that stressed plants are more vulnerable to aphid attack. Consequently, the increased instance of soil contamination and the high use of nitrogen based fertilisers in agriculture is advantageous to aphid populations.

Effects on plants do not seem to affect aphid consumption by ladybirds, in the present study, no significant relationship was found between the mass of aphids consumed by ladybirds during the feeding trial and the Cu treatment of the soil. Meaning that Cu contamination should not affect biological control. However, this was a short term, no-choice feeding trail and affects could still be apparent in the field. More investigations would be required to access development and fecundity in ladybirds reared on plants with increased heavy metals. Further chemical analysis on the adult ladybirds would be required to investigate any biochemical changes within the adult ladybirds.

All ecosystems are underpinned by soil characteristic; soils need to be viewed holistically to maintain these characteristics to ensure long term soil functioning. The acceleration of climate change brings uncertainty to species distribution and ecosystem functioning, more investigations are needed to fully understand the complex interactions between metals and soil functioning in relation to the corresponding ecological system.

The evidence available suggests that there might be critical levels of heavy metals for agri-ecosystems, across a range of soil types, climates and farm management practices. Considerably more quantitative investigation would be required to establish this clearly. The debate on heavy metals in soils will continue, if for no other reason than it as an indicator for soil quality or productive capabilities of the soil. However, the critical levels of heavy metals in soils will need further investigation if it is to be widely accepted as a potential risk to soil health and corresponding ecosystem functioning, the implementation of legislation and the development of long term soil monitoring frameworks.

7.0 Recommendations for further work.

There is a need for additional research on mixtures of pollutants as compounds rarely produced in isolation and the interactions of contamination is not well understood. Studies into the presences of multiple heavy metals affecting trophic interactions will be more meaningful in calculating the maximum safe levels of heavy metal exposure in agricultural soils and allow predictions into the impacts of pollution on bottom-up food webs interactions.

Any investigations should also include increased concentrations of heavy metals to asses if there is a plateau response or consequence of particular combinations of heavy metals. This may be important in reducing the exposure of heavy metals in the environment but also preventing the transference of heavy metals into the human food chain which has wider implications in developing countries. It could also provide a useful scale in the assessment of agricultural land across the UK and Europe.

From an entomological stand point further research need to be conducted on the effect of heavy metals on the life cycles of both pest and predator species and the ecological interactions between the two. As many insects have complex life stages assessing the development, growth, fecundity and survival rates of insects could provide important information into their control or provision of biological control.

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