PLANT GROWTH, BIOCHEMICAL CHARACTERISTICS AND HEAVY METALS CONTENTS OF *MEDICAGO SATIVA* L., *BRASSICA JUNCEA* (L.) CZERN. AND *CICER ARIETINUM* L.

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Abstract

The present research was conducted to study heavy metal accumulation and other physiological and biochemical parameters in three different field crops i.e. Alfalfa, *Brassica juncea* and Chickpea. The samples were collected from the field-grown crops at Malakandher Research Farm of Khyber Pakhtunkhwa Agricultural University Peshawar. Plant materials were analyzed for different physiological and biochemical parameters along with the determination of heavy metal accumulation by the collected samples of *Medicago sativa* L. *Brassica juncea (*L.) Czern. and *Cicer arietinum* L. . The data revealed that maximum plant fresh weight was recorded by Chickpea while minimum plant fresh weight was noted for *Brassica juncea*. Maximum plant dry weight was noted for Alfalfa and minimum plant dry weight was produced by Chickpea. Higher concentration of proline was noted in *Brassica juncea* while Chickpea produced lowest proline concentration. Maximum DNA concentration was noted in Chickpea while minimum DNA concentration was noted in Chickpea. Chickpea had the highest DNA purity. Minimum DNA purity was noted in *Brassica juncea.* Highest concentration protein was recorded by *Brassica juncea* while lowest protein concentration was noted for Chickpea. The data also showed that Chickpea recorded maximum Cd concentration. Minimum Cd concentration was noted in Alfalfa. Highest concentration of Cr was noted in Chickpea. Minimum Cr concentration was recorded by Alfalfa. Maximum levels of Pb was accumulated by Chickpea. Minimum levels of Pb were noted in *Brassica juncea*.

Introduction

The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). “Heavy metals” is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3 or more, greater than water (Hawkes, 1997). However, being a heavy metal has little to do with density but concerns chemical properties. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements (Farlex, 2005).

The bioavailability of metals in soil is a dynamic process that depends on specific combinations of chemical, biological, and environmental parameters (Panuccio *et al*., 2009). Soil management can also change its physical, chemical, and biological characteristics, and as a result, different responses by biological activities to heavy metal toxicity can be observed. Also, the activities of microorganisms that promote plant growth can be altered by high concentrations of metals (Wani *et al*., 2007). At high concentrations, all heavy metals have strong toxic effects and are regarded as environmental pollutants (Chehregani *et al*., 2005). Heavy metals are potentially toxic for plants: phyto-toxicity results in chlorosis, weak plant growth, yield depression, and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and, in leguminous plants, a reduced ability to fixate molecular nitrogen. Soil pollution with heavy metals will lead to losses in agricultural yield and hazardous health effects as they enter into the food chain (Schickler and Caspi, 1999). In heavy-metal-polluted soils, plant growth can be inhibited by metal absorption. However, some plant species are able to accumulate fairly large amounts of heavy metals without showing stress, which represents a potential risk for animals and humans (Oliver, 1997). Heavy metal uptake by crops growing in contaminated soil is a potential hazard to human health because of transmission in the food chain (Fries *et al*., 2006). There is also concern with regard to heavy metal transmission through natural ecosystems (Walker *et al*., 2003).

Phytoremediation is a process of metal uptake and accumulation by different plants depend on the concentration of available metals in soils, solubility sequences and the plant species growing on these soils (Kafka and Kuras 1997).

Cadmium, a heavy metal, widely used in industries, is considered as a human carcinogen. Cadmium (Cd), being a highly toxic metal pollutant of soils, inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animal and human health (Di-Toppi and Gabrielli, 1999). The application of sewage sludge, city waste, and Cd-containing fertilizers causes the increase of Cd content in soils (Williams and David, 1973). The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis (Padmaja *et al*., 1990). Excessive amount of Cd may cause decreased uptake of nutrient elements, inhibition of various enzyme activities, induction of oxidative stress including alterations in enzymes of the antioxidant defence system (Sandalio *et al*., 2001).

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Chromium (Cr) was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Vauquelin. It is a transition element located in the group VI-B of the periodic table with a ground-state electronic configuration of Ar 3d54s1. The stable forms of Cr are the trivalent Cr (III) and the hexavalent Cr (VI) species, although there are various other valence states which are unstable and short-lived in biological systems. Cr (VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate ($CrO_4{}^{-2}$) or dichromate ($Cr_2\,O_7{}^{-2}$) oxyanions. Cr (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments. Contamination of soil and ground water due to the use of Cr in various anthropomorphic activities has become a serious source of concern to plant and animal scientists over the past decade. Cr, in contrast to other toxic trace metals like cadmium, lead, mercury and aluminum, has received little attention from plant scientists. Its complex electronic chemistry has been a major hurdle in unraveling its toxicity mechanism in plants. The impact of Cr contamination in the physiology of plants depends on the metal speciation, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system. Cr toxicity in plants is observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis (Nriagu 1988).

Lead is among those heavy metals, which have no known biological function. Nevertheless, numerous investigations show that plants can accumulate lead via root and shoot, and that the lead concentrations in plant tissues are significantly related to the lead levels in environment. Excessive lead accumulated in plant tissue can be toxic to most plants, leading to decrease in seed germination, root elongation and biomass, inhibition of chlorophyll biosynthesis, as well as cell disturbance and chromosome lieson (Xiong 1997). In lead and other heavy metal-contaminatad sites, vegetation structure and biodiversity are usually reduced, barren patches of soil occur, and trees are sparse or absent (Wickland 1990). Lead has been listed as a potential carcinogen in the EPA Toxic Release Inventory (TRI). Inhalation and ingestion are the two routes of exposure, and the effects from both are the same. Pb accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. The gastrointestinal tract, kidneys, and central nervous system are also affected by the presence of lead. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk. Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead. (Wuana and Okieimen. 2011).

Brassica juncea belongs to the family Brassicaceae. *Brassica juncea* also known as mustard greens, Indian mustard, Chinese mustard and leaf mustard, is a species of mustard plant. Indian mustard (*Brassica juncea)* is a fast growing plant, which produces a high biomass even in heavy metal polluted soils. So far this plant species has been used in studies of the effects of heavy metals like cadmium (Anjum *et al*. 2008) and arsenic (Gupta *et al*. 2009) stresses on plants. Recently, Indian mustard plants (*Brassica sp*.) have been reported to have high potential to tolerate and accumulate high quantities of potentially toxic trace elements and can be used for phytoremediation of metal-polluted soils (Schafer *et al*. 1997).

The chickpea, *Cicer arietinum L*. (*Fabales: Fabaceae*), is one of the most important grain-legume crops in the world (Knights *et al*. 2007). Chickpea is considered a good source of proteins and carbohydrates. According to the Food and Agriculture Organization (FAO) statistics, cultivated chickpea is in the first rank, with about 10,671,503 ha cultivated, among cool season food legumes in the world.

The presence of nutrients, however, allows FA (Fly ash) utilization for agricultural purposes to fortify crops and cereals with nutrients, such as Fe, Se, and Zn. Leguminous crops are reported to be tolerant to many heavy metals. Chickpea is an important source of protein and many amino acids. Chickpeas are cultivated in semi-arid regions of India on a large scale. Although a lot of works have been done on the use of FA as a soil amender to boost up crop production, there is a dearth of studies dealing the anti-oxidative defense system in the crops in response to FA addition the growth, yield, level of metals, and anti-oxidative response in this economically important crop chickpea grown in FA treated soil (Pandey *et al*. 2010).

Alfalfa (*Medicago sativa* L.) is one of the most important legumes used in agriculture. Alfalfa is the most cultivated legume in the world with the US as the lead producer, but considerable area is found in Argentina (primarily grazed), Australia, South Africa, and the Middle East. Alfalfa has a wide range of adaptation and can be grown from very cold northern plains to high mountain valleys, from rich temperate agricultural regions to Mediterranean climates and searing hot deserts. Alfalfa is a potential source of biomaterials for the removal and recovery of heavy metal ions (Gardea-Torresdey *et al*. 2000).

Review of Literature: Haribabu *et al*. (2011) reported that the Contamination of the environment by toxic metals poses a threat for "Man and biosphere", reducing agricultural productivity and damaging the health of the ecosystem. Phytoextraction has emerged as a novel approach to clean up metal-polluted soils in which plants are used to transfer toxic metals from soils to shoots. During the accumulation of heavy metal, the antioxidant defense system helps the plant to protect itself from the damage caused by heavy metals. Antioxidants are substances that protect itself from damage caused by oxidation. *Brassica juncea* was exposed to the sublethal and half of sublethal concentration of copper and cadmium spiked soils and the antioxidant factors were investigated. The results showed that biochemical factors, vitamins and minerals, antioxidant activity and catalase values were decreased and proline, SOD and reduced glutathione values were increased compared to garden (control) soil.

Dasgupta *et al*. (2011) concluded that intensive industrial activity has resulted in contamination of soils with high concentrations of heavy metals and toxic elements, potentially bioaccumulated in crop and causing serious health and socio-economic problems. Soil pollution not only leads to pollution of water resources but also restricts the use of a site or can lead to soil degeneration. The present study was considered to take the advantage of bioaccumulative nature of heavy metals for soil remediation. The experimentation involves the usage of Chickpea for the phytoextraction of Pb and Cr. The seeds were sown in artificially contaminated soil separately with 25, 50, 75, 100 and 150 mg/Kg Cr and 50, 100, 200, 400 and 600 mg/Kg Pb. The plants were allowed to grow for a period of 30 days and bioaccumulation was analyzed at interval of 10 days. Higher bioaccumulation was observed for higher heavy metal concentration and longer growth periods. An attempt has been made in the present paper to compare the extent of soil cleanup with time.

Poniedziałek *et al*. (2010) performed an experiment which was carried out in 1999-2001 at the University of Agriculture in Krakow (Poland) to study the capacity of nine crops (red beet, field pumpkin, chicory, bean, barley, white cabbage, maize, alfalfa, and parsnip) to remove cadmium (Cd), lead (Pb) and zinc (Zn) from different soil horizons (0-20, 20-40, 40-60 cm). The content of exchangeable Cd, Pb, and Zn decreased along with the depth in the soil profile. Red beet cultivation reduced the exchangeable Cd content in the soil by 10.3% and by 8.6% in field pumpkin, barley and maize cultivation in the 0-20 cm horizon. White cabbage and maize decreased Cd in the 20-40 cm horizon by 40.0 and 28.8%, respectively. White cabbage was found to be the most effective in removing Pb from the soil profile. Common bean, maize, and alfalfa reduced exchangeable Pb in two upper horizons of the soil: 0-20 and 20-40 cm. After field pumpkin cultivation, the decrease in Pb contamination in the 0-20 cm horizon was 7.4%. White cabbage and chicory reduced exchangeable Zn content in the surface horizon by 21.5 and 14.1%, respectively. According to their efficiency in metal reduction, maize and red beet may be indicated as potential removers of Cd, cabbage and field pumpkin, of Pb, and cabbage, of Zn.

Shafi *et al*. (2010) examined the influence of Cd and Hg on Chlorophyll 'a', Chlorophyll 'b', total chlorophyll and proline of Chickpea plants was evaluated grown with or with nitrogen. Cadmium and mercury treatments at 10 and 25 mol/L affected chlorophyll and proline content of gram as compared to control. However, the addition of nitrogen (5 mM/L) somehow minimized the effect of heavy metals. Cadmium and mercury at 10 mol/L produced significant effect on chlorophylls. While higher concentrations (25 mol/L) significantly reduced chlorophyll content of plants. Nitrogen increased the chlorophyll content of metal treated plants. The proline content of plants was increased under Cd and Hg treatments (10 and 25 mol/L). In this case the additional nitrogen decreased proline content of plants treated with Cd and Hg.

Gualab *et al*. (2010) studied the effects of soil contamination by heavy metals are studied by a mathematical interaction model, validated by experimental results. The model relates the dynamics of uptake of heavy metals from soil to plants. The model successfully fitted the experimental data and made it possible to predict the threshold values of total mortality. Data are taken from soil with Cd, Cu and Zn treatments for alfalfa, lettuce, radish and Thlaspi caerulescens, measuring the concentrations in the aboveground biomass of plants. At low concentrations, the effects of heavy metals are moderate, and the dynamics seem to be linear. However, increasing concentrations exhibit nonlinear behaviors.

John *et al*. (2009) studied plant growth, pigment concentration, biochemical parameters and uptake of heavy metals were determined for *Brassica juncea L.* in response to cadmium and lead stress. The plant exhibited a decline in growth, chlorophyll content and carotenoids with Cd and Pb but Cd was found to be more detrimental than Pb treatment in *B. juncea*. The protein content was decreased by Cd (900 µM) to 95% and 44% by Pb (1500 µM) at the flowering stage. Proline showed increase at lower concentrations of Cd and Pb but at higher concentrations it showed decrease. More accumulation of Cd and Pb was observed in roots than shoots in B. juncea. Cd was found to be more accumulated than Pb but higher concentrations of Pb hampers the Cd absorption.

Cu *et al*. (2008) carried out an experiment in the greenhouse conditions with *Brassica juncea* L. grown on alluvial soils that had previously been contaminated at different concentrations of Cu. The main purposes of the research were to determine the effects of Cu and phosphorus applications on plant growth and Cu uptake by *Brassica juncea L.* Mature plants were harvested for the Cu accumulation analysis. The soil samples from each growing pot were extracted by HNO_3 $0._{43}$N in order to determine the content of $Cu2^+$ mobilization in soil, while the plant samples were acid digested for determining the total Cu concentration. Atomic Absorption Spectroscopy (AAS) was employed to determine Cu concentrations in soils and plant samples. The results showed that adding Cu to soils has strong effects on *Brassica juncea L.* growth and the uptake rate of Cu by the plants. The height and the biomass of plants were reduced dramatically by 36% and 53% respectively at the rate of 200 ppm Cu. In addition, phosphorous fertilizer also effectively improved plant growth and reduced Cu concentrations in plant of *Brassica juncea.* At the application rate of 100 kg P2O5/ha, the height and biomass of plant were increased to 30% and 31% respectively, and the Cu content in plants of *Brassica juncea* was reduced by 14% comparing with the control samples.

Turan *et al*. (2007) reported the use of plants to remove heavy metals from soil (phytoremediation) is expanding due to its cost-effectiveness as compared to conventional methods and it has revealed a great potential. Since contaminants such as Pb or Cd have a limited bioavailability in the soil, methods to facilitate their transport to the shoots and roots of plants are required for successful phytoremediation. The objective of this study was to investigate the effects of addition of different rates (0, 3, 6 and 12 mmol/kg) of ethylene diaminetetraacetate (EDTA) on heavy metal availability in soils contaminated with 50 mg/kg Cd ($CdCl_2$), 50 mg/kg Cu ($CuSO_4$), 50 mg/kg Pb [$Pb(NO_3)_2$] and 50 mg/kg Zn ($ZnSO_4$), and on the capacity of canola (*Brassica napus L.*) and Indian mustard (*Brassica juncea L.)* plants to uptake Cu, Cd, Pb and Zn in a growth chamber. Results indicated that EDTA application increased heavy metal availability and uptake by plants. Significant differences were obtained in both species and plant parts. As for plant species tested, canola was more effective in the uptake of Cu, Cd, Pb and Zn. Root heavy metal uptake of both species was higher than shoot heavy metal uptake.

Atici *et al*. (2005) concluded that the changes that take place in phytohormone contents in germinating chickpea (*Cicer arietinum* cv. Aziziye-94) seeds in response to heavy metal stress. For this aim, endogenous abscisic acid (ABA), gibberellic acid (GA3), zeatin (Z) and zeatin riboside (ZR) contents were followed for 24, 48 and 72 h in chickpea seeds germinating at the concentrations of 0.1, 1.0 and 5.0 mM Pb or 0.1, 1.0 and 10 mM Zn. The results showed that Pb and Zn significantly delayed and impeded the germination of chickpea seeds. The negative effect of Pb on germination was higher than that of Zn. Further, Pb increased ABA and Z contents while decreased GA3 content in the germinating seeds. The high concentrations of Zn (1.0 and 10 mM) decreased contents of Z, ZR and GA3 while 0.1 mM Zn increased the content of the same hormones. The ABA content was enhanced by Zn in all concentrations used.

Arun *et al*. (2005) studied that chromium is considered as a serious environmental pollutant. Contamination of soil and water by chromium (Cr) is of recent concern. Toxicity of Cr to plants depends on its valence state: Cr (VI) is highly toxic and mobile whereas Cr (III) is less toxic. Since plants lack a specific transport system for Cr, it is taken up by carriers of essential ions such as sulfate or iron. Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves, which may affect total dry matter production and yield. Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition. Metabolic alterations by Cr exposure have also been described in plants either by a direct effect on enzymes or other metabolites or by its ability to generate reactive oxygen species, which may cause oxidative stress. The potential of plants with the capacity to accumulate or to stabilize Cr compounds for bioremediation of Cr contamination has gained interest in recent years.

Gupta *et al*. (2004) reported that phytochelatin-related peptides were analyzed in chickpea plants exposed to six different heavy-metal ions. Cadmium and arsenic stimulated phytochelatin and homophytochelatin synthesis in roots but other metals did not. These metals, however, caused an overall increase in the precursors, glutathione, homoglutathione and cysteine. These changes may be different biochemical indexes for heavy-metal contamination.

EI-Kherbawy *et al*. (1989) studied the interaction between soil pH and inoculation with rhizobia and vesicular-arbuscular mycorrhizae (VAM) was studied in an industrially polluted soil contaminated with high levels of Zn and Cd. A silt loam soil (pH 6.7) was amended with $Ca(OH)_2$ or elemental S to adjust the soil pH to 4.3, 5.3, 6.0, and 7.2. Alfalfa (*Medicago sativa L.*) was planted in each treated soil subsequently inoculated with *Rhizobium meliloti* and/or a mixed VAM spore population. Alfalfa growing in soils at a pH of 4.3 and 5.3 failed to survive as a result of soil acidity and heavy metal toxicity. At the three higher pH values, growth and foliar N and P were significantly increased by inoculation with rhizobia or VAM. The greatest increase was observed when both VAM and rhizobia were inoculated together into the soil. With a soil pH of 6.0 and 6.7, the available heavy metal concentration in the soil was high and the VAM significantly decreased heavy metal uptake from these soils. The foliar concentration of Zn was reduced from 455 - 306 gg g^{-1} by inoculation with VAM (pH 6.0). At the highest soil pH (7.2), however, available heavy metal concentrations were generally lower and VAM significantly increased the heavy metal uptake. The influence of VAM on heavy metal uptake thus appears to be partly a function of the available heavy metal content in the soil.

Materials and Methods

The present research was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE) Khyber Pakhtunkhwa Agricultural University Peshawar during 2011. The aim of the study was to investigate heavy metal accumulation in Alfalfa, *Brassica juncea* and Chickpea and other physiological and biochemical parameters affected by (Pb, Cd and Cr). For this purpose Alfalfa, *Brassica juncea* and Chickpea were collected from the field grown crops at Malakandher Research Farm of Khyber Pakhtunkhwa Agricultural University Peshawar. Plant materials were analyzed for different physiological and biochemical parameters along with the determination of heavy metal accumulation by the collected Alfalfa, *Brassica juncea* and Chickpea. The following parameters were studied during the course of the study.

Plant materials: Three crops i.e; Alfalfa, *Brassica juncea* and Chickpea were used in the experiment, and following parameters were studied:

1. Plant fresh weight
2. Plant dry weight
3. Proline content
4. Protein content
5. DNA quantification and purity
6. Heavy metals (Pb, Cd and Cr)

1. Plant fresh weight: The fresh weight of plant was determined immediately after harvesting of plants with help of electronic balance.

2. Plant dry weight: After taking fresh weight, plant samples were dried in oven at 80 ^{o}C for three days to record plant dry weight.

3. Proline Content: Hundred mg of frozen plant material were homogenized in 1 ml of sterilized iron free water, the debris was removed by centrifugation at 5000 rpm. Proline was measured as describe by Bates *et al*. (1973) with minor modification. 250 µl of the extract was reacted with 1 ml of Acid Ninhydrin and 1ml of Glacial Acetic Acid. The mixture was then placed in water bath for 1hour at 100 ^{o}C, and the reaction was terminated in an ice bath. The reaction mixture was mixed with 4ml of Toluene and its Optical Density (OD) was measured at 520 nm. The amount of Proline was determined from standard curve.

4. Protein Extraction and Quantification: Protein was extracted by grinding about 100-800 mg lyophilized plant material pre-cooled mortar and pestle. The slurry was homogenized with 2ml buffer containing 100 mM Tris-HCl (pH 6.8), 1% SDS and 0.1% β-merceptoethanol and centrifugate at 15000 rpm for 10 minutes at 4 ^{o}C. The supernatant was collected and protein was quantified through Bradford method (Bradford, 1976) using Bovine Serum Albumin as standards.

5. Genomic DNA Extraction from leaves using 2X CTAB DNA Extraction Buffer

- First washed mortar and pistal and kept them in hot air oven for drying.
- Placed the explants inside it, cover it with aluminium foil and placed it at –80 ^{o}C for at least 2 hours.
- Grinded the leaf samples into fine powder and took it into the eppendorf tube.
- Transfer the grinded samples (100-200 mg) to 500-600 µl pre-warmed DNA 2X CTAB extraction buffer.
- Then put the sample in water bath at 60 ^{o}C for 1hour with gentle stirring.
- Take out the sample from water bath and 0.6 vol. of chloroform-isoamylalcohol (24:1) was added and mixed by shaking for 15 min.

- Centrifuge at 15,000 rpm for 10 min.
- Transfer the supernatant to other tube.
- Then add 0.6 vol. of isopropanol or twice volume of absolute alcohol to precipitate the DNA.
- And centrifuge shortly to pellet the DNA i.e 12,000 rpm for 10 min.
- Wash the pellet with 70% ethanol and place the tubes upside down on the tissue paper in LFU for 1 hour or dry overnight (cover with parafilm with tiny holes).
- Dissolve the dried DNA in 30 µl ddH2O.

6. Procedures for heavy metal analysis: Samples collected were dried at 80 °C for 48 hours and then finely grinded by electric grinder. Then the dried and crushed shoot sample (1 g) was prepared for atomic absorption spectrophotometer analysis. For this purpose samples were digested with 15 ml of concentrated nitric acid overnight. Digested samples were then heated to 250 °C till when white fumes appeared, and the heating was continued for another 1 hour. The samples were then cooled down to room temperature and diluted to 25 ml with distilled water and then filtered. Concentration of Pb, Cr and Cd was determined by atomic absorption spectrophotometer.

Results and Discussion

The present study describes the various physiological, biochemical and heavy metal concentrations of three different field crops i.e. Alfalfa, *Brassica juncea* and Chickpea. These parameters are presented and discussed below.

Physiological parameters: Data concerning plant fresh and dry weight is presented in Table 2. The data indicated in Table 2 revealed that maximum plant fresh weight of 8.45 g was recorded by Chickpea followed by Alfalfa with plant fresh weight of 8.07 g. Similarly, minimum plant fresh weight of 8.04 g was noted for *Brasscia juncea*. This difference may be due to differences in their genetic make up. Data recorded for plant dry weight as indicated in Table 2 revealed that maximum plant dry weight of 1.26 g was noted for Alfalfa followed by *Brassica juncea* 1.24 g plant dry weight while minimum plant dry weight of 1.12 g was produced by Chick pea (Table 2).

Table 1. Composition of 2X CTAB DNA extraction buffer.

Preparation of 2X CTAB DNA Extraction Buffer	
CTAB	**2%**
NaCl	**1.4 M**
EDTA	**20 mM (pH 8)**
Tris-HCl	**100 mM**
β-mercaptoethanol	**2µl/ml of buffer**

Table 2. Physiological and biochemical characters of Alfalfa, *Brassica juncea* and Chickpea.

Sample Name	Plant Fresh Wt. (g)	Plant Dry Wt. (g)	Proline (µg/g)	Protein (mg/ml)	DNA (mg/ml)	DNA Purity
Alfalfa	8.07	1.26	0.005	3.530	27.0	0.667
Brassica juncea	8.45	1.12	0.031	4.838	9.0	0.268
Chickpea	8.04	1.24	0.004	2.680	30.0	0.910

Table 3. Heavy metal conc. (µg/g) of Alfalfa, *Brassica juncea* and Chickpea.

Sample Name	Cadmium (Cd)	Chromium (Cr)	Lead (Pb)
Alfalfa	0.775	37.95	36.9
Brassica juncea	1.275	70.325	33.5
Chickpea	1.3	82.85	40.6

Biochemical parameters: Different biochemical characteristics investigated were proline, protein and DNA concentration of different field crops i.e. Alfalfa, *Brassica juncea* and Chickpea. (Table 2). It is clear from the data shown Table 2 that higher concentration of 0.031 µg/g fresh weight proline was noted in *Brassica juncea* followed by Alfalfa with 0.005 µg/g while Chickpea produced lowest proline concentration of 0.004 µg/g fresh weight. Data regarding DNA concentration and quality is shown in Table 2. The data revealed that maximum DNA concentration was noted in Chickpea 30.0 mg/ml followed by Alfalfa with DNA concentration of 27.0 mg/ml. The data indicated in Table 2 further suggested that minimum DNA concentration of 9.0 mg/ml was noted in *Brassica juncea*. It is also clear from the data shown in Table 2 that Chickpea had the highest DNA purity 0.910 followed by Alfalfa 0.667. Minimum DNA purity was noted in *Brassica juncea* 0.268. Data concerning protein concentration is also indicated in Table 2. The results revealed that highest concentration of 4.838 mg/ml protein was recorded by *Brassica juncea* followed by Alfalfa with 3.530 mg/ml while lowest protein concentration of 2.680 mg/ml was noted for wheat variety Chickpea (Table 2).

Heavy Metal concentration: Table 3 presents data regarding different heavy metal concentration in three different field crops i.e. Alfalfa, *Brassica juncea* and Chickpea collected from Malakandher Research Farm of KPK Agricultural University Peshawar. The data showed that Chickpea recorded maximum Cd concentration (1.3 µg/g) followed by *Brassica juncea* (1.275 µg/g). Minimum Cd concentration was noted in Alfalfa (0.775 µg/g). The data shown in Table 3 further revealed that highest concentration of Cr was noted in Chickpea (82.85 µg/g) followed by *Brassica juncea* with Cr concentration of (70.325 µg/g). The data also indicated that minimum Cr concentration of (37.95 µg/g) was recorded by Alfalfa. The data regarding Pb levels revealed that maximum levels of Pb was accumulated by Chickpea (40.6 µg/g) followed by Alfalfa with Pb levels of (36.9 µg/g). The data further showed that minimum levels of Pb were noted in *Brassica juncea* (33.5 µg/g) (Table 3).

Conclusion

The present study investigates various physiological, biochemical and heavy metal concentrations of three different field crops i.e. Alfalfa, *Brassica juncea* and Chickpea. Maximum plant fresh weight was recorded by *Brassica juncea* followed by Alfalfa. Similarly, minimum plant fresh weight was noted for Chickpea. Maximum plant dry weight was noted for Alfalfa followed by Chickpea while minimum plant dry weight was produced by *Brassica juncea*. Higher concentration of proline was noted in *Brassica juncea* followed by Alfalfa while Chickpea produced lesser proline concentration. Maximum DNA concentration was noted in Chickpea followed by Alfalfa while minimum DNA concentration was noted in *Brassica juncea*. Chickpea had the highest DNA purity followed by Alfalfa. Minimum DNA purity was noted in *Brassica juncea*. The results revealed that highest concentration protein was recorded by *Brassica juncea* followed by Alfalfa while lowest protein concentration was noted for wheat variety Chickpea.

The data showed that Chickpea recorded maximum Cd concentration followed by *Brassica juncea*. Minimum Cd concentration was noted in Alfalfa. Highest concentration of Cr was noted in Chickpea followed by *Brassica juncea*. Minimum Cr concentration was recorded by Alfalfa. The data regarding Pb levels revealed that maximum levels of Pb was accumulated by Chickpea followed by Alfalfa. Minimum levels of Pb were noted in *Brassica juncea*.

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