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Protein Level and Heavy Metals (Pb, Cr, and Cd) Concentrations in Wheat (*Triticum aestivum*) and in Oat (*Avena sativa*) Plants

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ABSTRACT: The aim of the study was to investigate heavy metal accumulation in wheat (*Triticum aestivum*) and oat (*Avena sativa*), and other physiological and biochemical parameters affected by these heavy metals. The data revealed that maximum plant fresh weight and plant dry weight was recorded for oat and minimum plant fresh weight and plant dry weight was noted for wheat (*Triticum aestivum*). The data also indicated that higher concentration of proline and DNA concentration was noted in wheat (*Triticum aestivum*) while lowest in oat (*Avena sativa*) *plant*. While DNA purity was highest in wheat (*Triticum aestivum*) and found lowest in oat (*Avena sativa*). Highest concentration of protein was recorded by wheat (*Triticum aestivum*) while lowest protein concentration was noted for oat (*Avena sativa*). The data further showed that wheat (*Triticum aestivum*) recorded maximum Cd concentration while minimum Cd concentration was noted in oat (*Avena sativa*). Highest concentration of Cr was noted in oat while minimum Cr concentration was recorded by wheat (*Triticum aestivum*). A maximum level of Pb was shown by oat (*Avena sativa*) while minimum levels of Pb were noted in wheat (*Triticum aestivum*). So oat (*Avena sativa*) plant is the higher accumulator of heavy metals i-e Cr and Pb while wheat (*Triticum aestivum*) accumulates Cd in highest concentrations.

KEYWORDS: Heavy metals, DNA, Protein content, Wheat, Oat.

1 INTRODUCTION

Wheat (*Triticum aestivum*) is usually the key feed along with a staple food intended for more than one third of the world population. It is sown about on 220 million hectares around the world wide with 564.6 million tons production; an average of 2500 kg grain feed every hectare. China sown wheat on about 30 million hectares, followed by the Russian Federation; India, the USA, Australia, Canada, Turkey and Pakistan. It is usually a new staple food plants associated with Pakistan, in addition to makes up about 36 % on the entire cropped region, 30 per cent on the importance added through important plants in addition to 76 % on the entire output associated with food grains. Among the wheat (*Triticum aestivum*) producing country, Pakistan stands at 10th place in terms of area (8.5 million hectares) and 59th in terms of yield (21.0 m ton) annually. Among the cereals, the particular wheat (*Triticum aestivum*) could be the majority of vantage and also crucial crop from the state. This specific edible foods crop accounts for about 37% from the cropped area of the state. The actual significant part of wheat (*Triticum aestivum*) throughout Pakistan is based on Punjab as well as Sindh. Nonetheless, the yield every hectare will be slightly increased throughout Sindh in comparison with Punjab as in [1].

Heavy metals tend to be conventially defined as elements with metallic qualities (ductility, conductivity, stability as cations for example) in addition to an atomic number _ 20. The most prevalent heavy metals tend to be Cd, Cr, Cu, Hg, Pb iand Zn. Crops harvested throughout heavy metal ripe substrata consume precious metal ions throughout varying levelsUptake is basically influenced through the availability associated with metals, which can be in turn based on both external (soil associated) and also internal (plant associated) components. In a limited amount of plant kinds a heritable tolerance or level of resistance occurs, which makes it possible for these plants to cultivate on metallic contaminated soil as in [2]. Soil remediation can be eradicating threat to help people as well as the environment from deadly metals. Numerous research dealing with heavy metal hyper accumulating plants, plus they include concluded that phyto extraction connected with metals has been a feasible remediation technological innovation to the decontamination connected with metal polluted soil. Recent research considering this feasibility connected with phyto extraction, demonstrated which both precious metal hyper accumulation and also excellent biomass assure are required to help to make the procedure efficient as in [3].

Phytoremediation is surely a rising technology that uses the use of larger plant life for that clean up polluted natural environment. Phyto extraction, the use of plant to be able to acquire poisonous metals from polluted soils, has emerged as a cost-effective, and environment friendly clean up alternate.

Industrial waste items certainly are a key supply of soil pollution as well as are derived from exploration industrial sectors, chemical industrial sectors, metal processing industrial sectors yet others. These waste items include a number of chemicals such as heavy metals and phenolics etc as in [4]. Use of industrial effluents along with sewage sludge on agricultural land has turned into a common exercise in India because of which these types of toxic metals can be transferred along with concentrated straight into plant tissues on the soil. These metals have damaging effects on the plants themselves and could become some sort of health risk to safety to person and pets. Above a number of levels in addition to on the small array, the heavy metals develop into toxic compounds as in [5]. Also, these metals adversely influence natural microbial populations resulting in disruption of vital ecological processes. Presently, microorganisms are increasingly being used seeing that potential bioindicators to the assessment of chemical risk towards the ecosystem. The effects of major metals about the growth of plants in addition to microorganisms are investigated by simply several individuals as in [6]. Abiotic stresses like heavy metal stress, air pollutants stress and so on negatively have an effect on processes related to biomass generation and grain yield in nearly all major field grown crops as in [7]. Each metal in addition to plant interacts in a very specific method, which is dependent upon several factors including type regarding soil, growth conditions as well as the presence of regarding other ions.

Phytoremediation continues to be utilised properly to remediate inorganic and organic pollutants with in the earth and from groundwater. Numerous plants, such as canola (*Brassica napus L*), oat (*Avena sativa*), and also barley (*Hordeum vulgare*), tolerate and also collects metals like selenium, copper, cadmium and zinc as in [8]. *Avena sativa* (oat) is a monocot, can be a yearly as well as perennial herb and is a member of family poaceae. However, oat developed into a well known winter season fodder crop from the rice system. One of many green winter months fodders, oats were being said to be additional palatable when compared with wheat or grain. The actual production rates involving green oats is usually more than two times in which involving green wheat in addition to oats can also be a multi cut plant as in [9].

Oat straw is sweet along with nourishing, and it is the key supplement with regard to tense exhaustion. Oat straw benefits inside expresses involving major depression, anxiety, failure for a person to completely focus, melancholy, along with weakness. It's a excellent tonic natural herb, can be taken over long periods of time and is also secure for all a long time. Oats are usually high in terms of iron, manganese, zinc, calcium mineral, magnesium, and also potassium and are also well suited for people healing coming from long-term or even debilitating health issues. Benefit of white oat (*Avena sativa*) possesses increased within the last few years, with a new steep increase in grown area to meet the market place demand. On account of health positive aspects, such for the reason that reducing influence of b-glucan on blood cholesterol levels, the crops have become integrated inside the human food chain as in [10]. The integration of white oats with production systems also allows a vast improvement of garden soil physical, chemical and biological properties, lowering of pests and conditions in additional crops and allelopathic weed control as in [11],[12]. A hydroponic screening of 22 grass species indicated that oat (*Avena sativa*) and barley (*Hordeum vulgare*) tolerated th high Cu, Cd and Zinc concentrations present in the solution and also accumulated elevated concentrations of these metals in the plant shoots as in [13].

2 OBJECTIVES

The present study was aimed to find a suitable plants species for use in cleaning up the soil in industrial regions.

3 MATERIALS AND METHODS

The aim of the study was to investigate heavy metal accumulation in wheat and oat, and other physiological and biochemical parameters affected by these heavy metals. For this purpose plants from wheat and oat were collected from the field grown crops at Malakandhere Research Farms of The University of Agriculture Peshawar. Plant materials were analyzed for different physiological and biochemical parameters along with the determination of heavy metals accumulation by the collected plants. The following parameters were studied during the course of the study.

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

3.1 PLANT FRESH WEIGHT AND PLANT DRY WEIGHT

Plant fresh weight has been registered by taking fresh weight regarding ten plants by using electric balance as well as the averages has been then determined. Plants obtained with regards to fresh weights data were being next dried with 80 ºC for 48 hours within oven as well as the dried out excess weight has been noted by making use of an electronic balance as well as averaged.

3.2 PROLINE CONTENT

Proline had been tested as in [14] with minor modifications. For this purpose, 100 mg associated with frozen plant material was homogenized inside 1ml of sterilized distilled water and the debris was removed simply by centrifugation at 5000 rpm. 250 µl of the extract was reacted with 1ml of acid Ninhydrin in addition to 1ml of Glacial acetic acid. The mixture was then positioned in a water bath for 1 hour at 100 ^{o}C, and the reaction was terminated in an ice bath. The reaction mix was mixed with 4 ml of toluene and its optical density was calculated at 520 nm. The amount of proline in unknown samples was determined from standard curve.

3.3 PROTEIN EXTRACTION AND QUANTIFICATION

Healthy proteins seemed to be extracted by means of grinding regarding 800 mg lyophilized plant material pre-cooled mortar and pestle. The slurry was homogenized with 2 ml buffer containing 100 mM Tris-HCl (pH 6.8), 1% SDS and 0.1% β-merceptoethanol and centrifuged with 15000 rpm for 10 minutes at 4^{o}C. The seemed to be collected and necessary protein seemed to be quantified via Bradford procedure making use of Bovine Serum Albumin as standards as in [15].

3.4 GENOMIC DNA EXTRACTION FROM LEAVES OF WHEAT AND OAT

Leaf samples were taken and grinded in to good powdered form and also transmitted that into eppendorf tubes. 100 mg of grinded leaf samples included with 600 µl pre-warmed DNA 2X CTAB extraction buffer (Table 1). After that 0. 6 volume of chloroform isoamylalcohol (24: 1) had been added in, mixed by shaking for 15 minutes and also centrifuged from 15000 rpm intended for 10 minutes. The supernatant had been transferred to clean tubes in addition to included 0. 6 amounts associated with iso-propanol to precipitate the DNA. The samples have been then centrifuged on 12000 rpm for 10 moments. Then the pellet had been cleaned together with 90%, 80% in addition to 70% ethanol and then dried by means of adding the tubes upside down for 10 minutes. The dried pellet had been then mixed inside distilled mineral water on 60 ºC inside water bath to help dissolution. DNA samples have been then located or stored on at -80 ºC until used. The samples have been then quantified as a result of UV-spectrophotometer on 260 nm and on 280 nm.

Table 1. Composition of 2X CTAB DNA extraction buffer

Composition 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

3.5 PROCEDURES FOR HEAVY METAL ANALYSIS

Samples collected were dried in at 80 °C with regard to 48 hrs and are next finely grinded through grinder. The dried as well as crushed shoot samples (1g) were next made up for atomic absorption spectrophotometer analysis. For this purpose, samples were then mixed having 15 ml concentrated nitric acid overnight. The samples were next warmed up for up to 250 °C until eventually when white fumes seemed, and the heating system was carried on with regard to one more hour. The samples were subsequently cooled off to room temperatures as well as diluted to help 25 ml with distilled water and were then filtered. Concentration of Pb, Cr as well as Cd had been determined by atomic absorption spectrophotometer in wavelengths of 283 nm, 357 nm as well as 228 nm respectively. Analysis of the soil before sowing revealed that the concentrations of Pb, Cr and Cd were 59.25, 28.75, 1.94 mg kg 1 respectively.

4 RESULTS AND DISCUSSION

The present research work describes the various physiological, biochemical and heavy metal concentrations of wheat and oat. These parameters are presented and discussed below.

4.1 PHYSIOLOGICAL PARAMETERS

Table 2 shows data regarding plant fresh and dry weight of wheat and oat. The data shown in Table 2 revealed that maximum plant fresh weight of 8.21 g was recorded by oat. Similarly, minimum plant fresh weight of 5.54 g was noted for wheat. This difference may be due to differences in their genetic make up. Data recorded for plant dry weight as shown in Table 2 indicated that maximum plant dry weight of 1.63 g was of oat plant recorded while minimum plant dry weight of 1.08 g was produced by wheat (Table 2).

4.2 BIOCHEMICAL PARAMETERS

The various biochemical parameters studied were proline, protein and DNA concentration of wheat and oat (Table 2). The data indicated in Table 2 showed, that higher concentration of 0.04 µg/g fresh weight proline was noted in wheat while oat produced proline concentration of 0.02 µg/g fresh weight. Data regarding DNA concentration and quality is indicated in Table 2. The result showed that maximum DNA concentration was noted in wheat (43.5 mg/ml) followed by oat with DNA concentration of 13.0 mg/ml. It is also clear from the data shown in Table 2 that wheat had the highest DNA purity (1.10) while minimum DNA purity was noted in oat (0.28). Data concerning protein concentration is indicated in Table 2. The results revealed that highest concentration of 5.82 mg/ml protein was recorded by wheat while lowest protein concentration of 3.76 mg/ml was noted for oat (Table 2).

Table 2. Physiological and biochemical characters of wheat, oat and barley

Genotypes	Plant Fresh Weight (g)	Plant Dry Weight (g)	Proline (µg/g)	Protein (mg/ml)	DNA (mg/ml)	DNA Purity
Wheat	5.54	1.08	0.04	5.82	43.5	1.10
Oat	8.21	1.63	0.02	3.76	13.0	0.28

4.3 HEAVY METAL CONCENTRATION

Table 3 presents data regarding different heavy metal concentration in wheat and oat collected from Malakandhere Research Farms of The University of Agriculture Peshawar. The data showed that wheat recorded maximum Cd concentration (2.55µg/g) and minimum Cd concentration was noted in oat (0.26 µg/g). The data in Table 3 further revealed that highest concentration of Cr was noted in oat (53.002µg/g) while minimum Cr concentration of 33.0 µg/g was recorded by wheat. The data concerning Pb levels revealed that maximum levels of Pb was shown by oat (40.10 µg/g) followed by wheat with Pb levels of 37.18 µg/g (Table 3).

Table 3. Heavy metal concentrations (µg/g) of wheat, oat and barley

Genotypes	Cadmium (Cd)	Chromium (Cr)	Lead (Pb)
Wheat	2.55	33.00	37.18
Oat	0.26	53.02	40.10

5 CONCLUSION

The present study investigates heavy metal uptake (Cd, Cr and Pb) by wheat and oat and their physiological and biochemical parameters. The data revealed that maximum plant fresh weight was recorded by oat and minimum plant fresh weight was noted for wheat. Maximum plant dry weight was recorded for oat while minimum plant dry weight was produced by wheat. The data also indicated that higher concentration of proline was noted in wheat while oat produced lowest proline concentration. Data regarding DNA concentration and quality showed that maximum DNA concentration was noted in wheat followed by oat with minimum concentration. Wheat had the highest DNA purity followed by oat.

Highest concentration of protein was recorded by wheat while lowest protein concentration was noted for oat. The data showed that wheat recorded maximum Cd concentration while minimum Cd concentration was noted in oat. Highest concentration of Cr was noted in oat while minimum Cr concentration was recorded by wheat. A maximum level of Pb was shown by oat while minimum levels of Pb were noted in wheat.

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SYED NOOR UL ABIDEEN

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