soil systems

MDPI

Article

Evaluating Potential Ecological Risks of Heavy Metals of Textile Effluents and Soil Samples in Vicinity of Textile Industries

Jaskaran Kaur [1], Sandip Singh Bhatti [1], Sartaj Ahmad Bhat [2,3], Avinash Kaur Nagpal [1], Varinder Kaur [4] and Jatinder Kaur Katnoria [1,*]

[1] Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, Punjab, India; jaskaranbot.rsh@gndu.ac.in or jaskarankaur0@gmail.com (J.K.); singh.sandip87@gmail.com (S.S.B.); avinash.botenv@gndu.ac.in or avnagpal@rediffmail.com (A.K.N.)
[2] Department of Environmental Sciences, Government Degree College, Khanabal, Anantnag 192101, Jammu and Kashmir, India; sartajbhat88@gmail.com
[3] River Basin Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[4] Centre for Advanced Studies, Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, Punjab, India; varinder_texchem@gndu.ac.in or varinder_gndu@yahoo.com
* Correspondence: jatinder.botenv@gndu.ac.in or jatinkat@yahoo.co.in or jkat08@yahoo.com

Abstract: The present study pertains to assessing the heavy metal (Cd, Cr, Co, Cu, Pb, and Zn) contents of untreated and treated effluents of two textile industries and agricultural soil samples in the vicinity of these industries located in Ludhiana, Punjab (India). The genotoxicity of the effluents samples was estimated using *Allium cepa* root chromosomal aberration assay. The exposure of *Allium cepa* roots to untreated effluents from both industries resulted in the reduction of mitotic index (MI) and increase in chromosomal aberrations in the root tip meristematic cells when compared to those that were exposed to the treated effluents indicating the significant genotoxic potential of untreated effluents. Risk characterization of soil sample was carried out by calculating the potential ecological and human health risks of heavy metals. The hazard index was observed to be less than 1, indicating there was no potential health risk of heavy metals in soil samples. Furthermore, bioaccumulation potential studies on plant species grown in the vicinity of these industries have shown that bioaccumulation factor (BAF) varied as *Ricinus communis* L. > *Chenopodium album* L. > *Cannabis sativa* L. with Co and Pb having maximum and minimum values, respectively.

Keywords: health risk assessment; *Allium cepa* root chromosomal aberration assay; bioaccumulation; genotoxicity; heavy metals; industrial effluents

Citation: Kaur, J.; Bhatti, S.S.; Bhat, S.A.; Nagpal, A.K.; Kaur, V.; Katnoria, J.K. Evaluating Potential Ecological Risks of Heavy Metals of Textile Effluents and Soil Samples in Vicinity of Textile Industries. *Soil Syst.* **2021**, *5*, 63. https://doi.org/10.3390/soilsystems5040063

Academic Editors: Matteo Spagnuolo, Paola Adamo and Giovanni Garau

Received: 18 June 2021
Accepted: 27 September 2021
Published: 9 October 2021

1. Introduction

Numerous obnoxious chemical agents continuously enter our environment due to various industrial, domestic, and other human activities. These chemicals have the tendency to pose threats to the survival of living beings, ultimately endangering the ecological balance [1–3]. The water pollution index on account of inorganic chemicals is considered to be one of the major indicators of environmental pollution, which has accelerated in past decades due to various anthropogenic activities, especially, agricultural practices and the discharges of effluents from industries into the natural water bodies. [4]. The release of huge quantities of treated, as well as untreated municipal wastes, to aquatic bodies has also become a problem in different developing countries [5]. Wastewater irrigation has been documented to cause the accumulation of heavy metals in agricultural soils and plants [6–9].

The contamination of water bodies due to genotoxic compounds like heavy metals and pesticides has been widely documented [10–12]. The presence of various unidentified and noxious toxicants possessing potential carcinogenicity has been widely demonstrated in various genotoxicity studies [13,14]. The reports on genotoxicity studies of various industrial wastewaters and other effluents have globally raised concern over the genotoxic and carcinogenic hazards of the contaminants present in the samples [1]. Since the chemical characterization alone cannot provide sufficient knowledge on their genotoxicity and potential hazard, different bioassays have been used to explore the same. Many bioassays have been effectively used to assess the genotoxicity of complex wastewaters and a number of bacterial and plant-based assays have been developed for the estimation of the genotoxic potential of water samples. Among these, the *Allium cepa* test takes a prominent position because it has a low chromosome number and large size of chromosomes [15].

Soil is an essential resource for sustaining two basic human necessities, that is, production of sufficient food and a clean environment by adsorbing different contaminants. However, certain plants grown on polluted land can uptake contaminants like heavy metals either as ions through their root system or by absorption through foliage, and they get accumulated in different plant parts such as in roots, stems, leaves, fruits, and grains [16]. Heavy metal contamination of soils is a very serious issue that has contributed significantly to the contamination of various food crops [3,17,18]. Although heavy metals exist in soils in natural concentrations (significantly low) deriving from parent rock materials, these trace amounts do not pose any harm to human health. However, anthropogenic inputs of wastewaters from various sources along with the dumped waste can significantly increase the heavy metal concentrations in soil [19,20]. Excessive levels of heavy metals in agricultural soils not only lead to the disorders of soil functions and crop growth but also, poses serious risks to human health by accumulating in food crops [21–24].

Potential human health risk (non-carcinogenic and carcinogenic) assessment has been recognized as an efficient tool for assessing risks of various pollutants in the environment and is essential for making decisions regarding regulations concerning pollution reduction in urban soil and minimizing human exposure to toxic pollutants [25–27]. Considering the ecological threats posed by contaminants in textile industry effluents, the present study was conducted to assess the effluents (treated and untreated) from two textile industries situated in Punjab, India for heavy metal contents, physico-chemical characteristics, and genotoxicity following the *Allium cepa* root chromosomal aberration assay. Heavy metal estimation and ecological risk assessment of the agricultural soil in the vicinity of these industries were also conducted. The study further focused on the evaluation of heavy metal bioaccumulation in three plant species viz., *Cannabis sativa* L., *Ricinus communis* L., and *Chenopodium album* growing in the vicinity of these industries, as well as the application of various pollution indices to determine the pollution level of analyzed heavy metals in the soil of study area.

2. Material and Methods

2.1. Collection of Samples

2.1.1. Textile Industrial Effluents

Untreated and treated effluents originating from two textile industries (Textile Industry A and Textile Industry B) being discharged into the Sutlej river, Ludhiana, Punjab, India, were selected for toxicity assessments. In the present study, the effluent samples from both textile industries were collected during March 2017. Effluent samples from the respective industries were collected in triplicate in clean bottles, brought to the laboratory, and stored at 4 °C until further analysis. The samples were coded as shown in Table 1. The physico-chemical analysis of the collected samples was carried out following standard protocols [28,29].

Table 1. Description of sample codes.

S. No.	Sample Code	Description of Sample
1.	AU	Untreated effluent sample collected from textile industry A
2.	AT	Treated effluent sample collected from textile industry A
3.	BU	Untreated effluent sample collected from textile industry B
4.	BT	Treated effluent sample collected from textile industry B
5.	SA	Soil sample collected from an agricultural field in the vicinity of industries A and B

2.1.2. Soil Sample

Soil samples in triplicate were taken from agricultural fields in the vicinity of the industries. For the collection of soil samples, the soil was dug to the depth of 20 cm [30]. Soil was collected from 4–5 parts of the field viz., east, west, north, south, and center and pooled to constitute the sample of the particular field. Approx. area of the agricultural field was 1000 sq. mts and situated 500 meters away from the main industrial units. Soil samples were stored in clean and airtight polyethylene bags. Soil samples were dried in the laboratory, cleaned by removing visible traces of leaves and other waste materials, homogenized, and sieved through a size 2 mm sieve for heavy metal analysis.

2.1.3. Plant Samples

Since there was no crop grown at the time of sampling in the agricultural field, the plant samples (leaves) of three wildly growing plant species viz. *Cannabis sativa* L. (Cannabaceae), *Chenopodium album* L. (Amaranthaceae), and *Ricinus communis* L. (Euphorbiaceae) on the boundaries of agricultural fields in the vicinity of industries were collected to explore their heavy metal bioaccumulation potential. The leaves were thoroughly washed using tap water followed by distilled water, oven-dried at 70 °C, grounded to a fine powder by pestle mortar, and stored in airtight polyethylene bags at 4 °C until further analysis.

2.2. Physico-Chemical Characteristics of Industrial Effluents and Soil

The soil extract (1:5 *w*/*v*) was prepared by adding 20 g of the collected soil sample in 100 mL of distilled water. This solution was kept in a mechanical shaker for 12 hours at room temperature and filtered through Whatman No. 1 filter paper [31]. The filtrate was termed soil extract and was used for further analysis of the physico-chemical parameters (pH, electrical conductivity, calcium, sodium, and magnesium). Total organic carbon of the soil was estimated using the dry combustion method [32]. A core measuring cylinder (100 ML) was used for bulk density (BD) estimation [33]. Soil texture was determined by the sieving and sedimentation method [34]. On the basis of size, different particles of soil were grouped as: sand: 0.5–2.00 mm; silt: 0.002–0.5 mm; clay: <0.002 mm. The analysis of the physico-chemical parameters (pH, temperature, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), total hardness, alkalinity, calcium, chloride, magnesium, sodium, and phosphate) of effluent samples was carried out following the standard protocols of the American Public Health Association [35,36]. The sodium content of both effluents and soil samples was measured using a Flame Photometer (Model-128; Make: Systronics). The pH of each effluent was measured using a pH meter (Model: µ pH system 361; Make: Systronics).

2.3. Heavy Metal Estimation

Heavy metal contents in collected samples were determined using the flame atomic absorption spectrophotometer (AAS) (Agilent 240 FS AA model), at variable/recommended wavelength of 228.80 nm for cadmium, 240.70 nm for chromium, 357.90 nm for cobalt, 324.80 nm for copper, 217.0 nm for lead, and 213.90 nm for zinc. Limits of detection (µg/L) for different metals were cadmium (1.5), cobalt (3), chromium (5), copper (1.2), lead (7), and zinc (1.6). The airflow rate was maintained at 13.50 L/min for all heavy metal determinations. The acetylene flow rate was set at 2.00 L/min for Cd, Co, Cu, Pb, and Zn, and at 2.90 L/min for Cr estimations while the lamp currents were set at 4.00 mA, 7.00 mA, 7.00 mA, 4.00 mA, 10.00 mA, and 5.00 mA for determination of Cd, Co, Cr, Cu, Pb, and Zn, respectively. All the glassware was thoroughly washed and oven-dried before use. Double distilled water and analytical grade reagents were used during the whole experiment. The standard solutions (1000 mg/L) of Agilent made for different metals were used to prepare solutions of varying concentrations as 0.5, 1, 1.5 (mg/L) for cadmium and zinc; 5, 10, 15 (mg/L) for chromium, lead, nickel, and cobalt; and 1, 3, 5 (mg/L) for copper using the serial dilution method. The accuracy (>95%) of the instrument was maintained throughout the experiment by thorough washing. For which, after every 10 sample readings, the standards were run to observe the accuracy of the instrument. Soil samples were digested using aqua regia, that is, a mixture of one part concentrated nitric (HNO_3) and three parts hydrochloric acid (HCl) following the method described by the authors of [37] with minor modifications. For this purpose, 1 g of finely ground soil sample was digested slowly with aqua regia on a hot plate in a fume hood till white fumes appeared, indicating the complete digestion of the soil sample. Plant sample digestion was carried out using a tri-acid mixture, that is, five parts of nitric acid (HNO_3) and one part of both perchloric ($HClO_4$) and sulfuric acid (H_2SO_4) as prescribed by Allen [38]. Only concentrated acids were used for both types of digestion. The digested soil and plant samples were filtered using Whatman No.1 filter paper and diluted with double distilled water up to a final volume of 50 mL.

2.4. Metal Bioaccumulation Factor (BAF)

In order to assess the accumulation of heavy metals from the soil in the agricultural fields in the vicinity of the industries into the three plant species (*Cannabis sativa* L., *Chenopodium album* L. and *Ricinus communis* L.), the bioaccumulation factor (BAF) was calculated. The bioaccumulation factor is commonly used to study the fate of different environmental contaminants in plants [39]. Ali et al. [40] documented that BAF is the ratio of the concentration of heavy metals in the crop to that in the soil. Accordingly, BAF was calculated using the following equation.

$$\mathrm{BAF} = \mathrm{C_{plant}}/\mathrm{C_{soil}} \quad (1)$$

where, C_{plant} stands for concentrations of heavy metal in plant leaves and C_{soil} stands for concentrations of heavy metal in soil.

2.5. Genotoxicity Assessment

The genotoxicity of both untreated and treated industrial effluents was determined using the *Allium cepa* root chromosomal aberration assay [41–43]. After the removal of primary roots of freshly purchased onion bulbs, bulbs were placed on Couplin jars containing distilled water (negative control) and industrial effluents for 48–72 h for rooting. The Couplin jars were kept in a BOD (Biochemical Oxygen Demand) incubator at 25 $\pm$ 2 °C until roots grew. Care was taken to fill the coupling jars with exposure media on a daily basis so that the onion bulbs were emersed in solution and the root primordia were under continuous exposure to the treatment. Distilled water was used as a negative control during the study. The onion bulbs after treatment were thoroughly washed. The root tips were plucked with forceps and put in a solution of glacial acetic acid and Ethanol in the ratio of 1:3 (Farmer's fluid). The root tips were squashed in aceto-orcein stain to prepare slides. At least five slides consisting of approximately 500 dividing cells were examined under a light microscope to calculate mitotic index (MI) and to score different types of aberrations for each sample. The chromosomal aberrations were categorized into physiological (outcomes of spindle inhibition) and clastogenic (formed due to damage of DNA) based on the descriptions given earlier [42,44].

2.6. Pollution Assessment

The degree of pollution of the agricultural soil in the studied area was evaluated by using several indices, like the geoaccumulation index (Igeo), contamination factor (CF), degree of contamination (Cd_{eg}), modified degree of contamination (mCd_{eg}), Numerow's pollution index (PI), pollution load index (PLI), potential ecological risk factor (ER_i), and the potential ecological risk index (RI). A brief description of these soil contamination indices is given in Table S1.

2.7. Human Health Risk Assessment

The model for human health risk assessment given by the United States Environmental Protection Agency (USEPA) was used to assess the non-carcinogenic and carcinogenic effects of environmental toxicants like heavy metals on humans. Due to behavioral and physiological differences in this study area, people were divided into two groups, that is, adults and children. Soil contaminants, that is, heavy metals, pose health risks to the human body mainly by three exposure pathways, which include ingestion, inhalation, and dermal contact. So, the carcinogenic and non-carcinogenic threat of these exposure pathways was calculated in the present study. The methodology used in the present study for human health risk assessment was based on the guidelines given by the US Environmental Protection Agency [28,45–48].

2.7.1. Exposure Assessment

To calculate the human exposure dose, the average daily intake (*ADI*) of heavy metals in soil for three exposure pathways (ingestion, inhalation, and dermal contact) is calculated as follows:

Ingestion pathway:

$$ADI_{ingestion} = \frac{C \times IR_{ig} \times EF \times ED \times CF}{BW \times AT} \quad (2)$$

Inhalation pathway:

$$ADI_{inhalation} = \frac{C \times IR_{ih} \times EF \times ED}{BW \times AT \times PEF} \quad (3)$$

Dermal contact pathway:

$$ADI_{dermal\ contact} = \frac{C \times SA \times SAF \times DAF \times EF \times ED \times CF}{BW \times AT} \quad (4)$$

where $ADI_{ingestion}$, $ADI_{inhalation}$, $ADI_{dermal\ contact}$ is the average daily intake (mg/kg day) via ingestion, inhalation, and dermal contacts, respectively. *C* is the concentration of analyzed heavy metals in soil samples (mg/kg); IR_{ig} is the ingestion rate (100 and 200 mg/day for adults and children, respectively) [28,45,48]; IR_{ih} is the inhalation rate (12.8 m^3/day for adults and 7.63 m^3/day for children) [28]; *EF* is the exposure frequency (365 days/year) [28,45]; *ED* is the exposure duration (30 years for adults and 6 years for children) [28]; *CF* is the conversion factor for soil (10^{-6} kg/mg) [48]; *BW* is the body weight (70 and 20 kg for adults and children, respectively) [28]; *AT* is the average exposed time (*EF* × *ED*) [28]; PEF is the particulate emission factor (1.36×10^9 m^3 /kg) [28]; *SA* is the skin exposed area for soil (4350 and 1600 cm^2 for adults and children, respectively) [28]; *SAF* is the skin adherence factor (0.7 mg/cm^2 for adults and 0.2 mg/cm^2 for children) [28,48]; and *DAF* is the dermal absorption factor (0.001) [28].

2.7.2. Non-Carcinogenic Risk Assessment

The hazard quotient (*HQ*) is characterized for non-carcinogenic hazards and is defined as the average daily intake by the toxicity threshold value, which is referred to as the chronic reference dose (*RfD*) in mg/kg-day of the specific heavy metal. *HQ* is computed as the ratio of the average daily intake (*ADI*) and a reference dose (*RfD*). The equitation of *HQ* is given as follows [28,45]:

$$HQ = \frac{ADI}{RfD} \quad (5)$$

where *HQ* is the hazard quotient, *ADI* is the average daily intake (mg/kg day) and *RfD* is the reference dose (mg/kg day) of heavy metals via ingestion, inhalation, and dermal contact pathways. The reference dose (*RfD*) of studied heavy metals is shown in Table S2.

Hazard index (*HI*) is a cumulative non-cancer health risk that can be evaluated by the sum of the *HQ* (hazard quotient) values of various exposure pathways. It can be calculated as the sum of non-carcinogenic hazard quotients for all contaminants [45] as follows:

$$HI = \sum HQi \quad (6)$$

where *HQi* is the non-cancer hazard quotient for the ith contaminants.

HI < 1 indicated no non-carcinogenic health, whereas *HI* > 1 risk indicated adverse non-carcinogenic health risk [28,49].

2.7.3. Carcinogenic Risk Assessment

The carcinogenic risk assessment is the incremental probability of an individual developing cancer over a lifetime as a result of exposure to the potential carcinogen like heavy metals [27,50]. Carcinogenic risk and total carcinogenic risks are determined as follows:

$$CR = ADI \times SF \quad (7)$$

$$TCR = \sum CR \quad (8)$$

where *CR* is the carcinogenic risk; *ADI* is the average daily intake (mg/kg day); *SF* is the cancer slope factor over a lifetime (mg/kg day). The cancer slope factor (*SF*) of studied heavy metals is shown in Table S2.

The values of carcinogenic risk (*CR*) ranging from 1×10^{-6} to 1×10^{-4} are considered as safe limit for human health [28,45], whereas higher *CR* values than the limit of 1×10^{-4} cause lifetime cancer risks to the human body [45,49].

2.8. Statistical Analysis

Student's *t*-test ($p \leq 0.05$) was applied to find significant differences between values of heavy metals and genotoxicity parameters like mitotic inhibition (MI), physiological aberrations (PA), clastogenic aberrations (CA), and total aberration (TA) for untreated and treated effluents of the same industry. Chi-square test ($p \leq 0.05$) was used to calculate the statistically significant differences between the values of genotoxicity parameters (MI, PA, CA, and TA) for effluents and the negative control. Statistical analysis was performed using Minitab version 14.0 (State College, PA, USA).

3. Results and Discussion

3.1. Physico-Chemical Characteristics of Industrial Effluents and Soil

Physico-chemical parameters of studied soil and industrial effluent samples are shown in Table 2. The mean pH of soil, that is, 8.02 was observed to be within the permissible limit of 6.5–8.5 and is alkaline in nature. Electrical conductivity (EC), which indicates the soil salinity, was also found to be within the prescribed limit. The studied soil sample had a sand content of 33.49%, silt content of 26.05%, and clay of 40.45%. The Ca, Mg and Na contents (mg/kg) of the soil sample were observed to be 120.24, 176.64, and 343.08, respectively. pH, the most significant parameter for the assessment of water quality, ranged from 6.67 to 8.90 and remained within the prescribed limits. Bulk density (BD) plays a vital role in the growth of plants as high BD can decrease the root penetration in soil. The mean bulk density (BD) of the studied soil sample was found to be 1.08. Organic matter content plays a chief role in the fertility of agricultural soils. Total organic carbon (TOC) in the present study was observed to be 2.22%. The pH of treated effluent of textile industry A (AT) was observed to be acidic while all other effluent samples showed basic pH. Dissolved calcium and magnesium in water are the two most common minerals that determine water hardness. Total hardness (mg/L) for AU, AT, BU, and BT was found to be 111.33, 151.33, 191.33, and 104.67, respectively. The calcium content in effluent samples ranged between 17.90–60.65 mg/L and magnesium content was seen in the range of 17.58–70.33mg/L. The order of chloride content (mg/L) was observed to be: 232.41 (AU) > 142.47 (AT) > 114.07 (BU) > 66.74 (BT). The electrical conductivity varied from 511µS/cm to 1908.67 µS/cm. The value of total suspended solids (mg/L) was found to be minimum for AU (106.67) and maximum for AT (585) while the content of total dissolved solids (mg/L) was found to be minimum for AT (201.67) and maximum for BU (3666.67). The value of alkalinity (mg/L) varied from 356.67 to 656.67; sodium content (mg/L) from 141.08 (BU) to 333.63 (AU); and phosphate content (mg/L) from 1.48 (AT) to 2.08(BU). The value of total solids (mg/L) was found to be in the order of BU (3893.33) > AU (3473.33) > BT (2000) > AT (786.67).

According to Paul et al. [51], the basic nature of the pH of the industrial effluents was because of the usage of scouring and bleaching agents along with other various chemicals like caustic soda, hydrogen peroxide, and soap while pulping the waste. Similar results were also reported by Ramamurthy et al. [52]. Alkaline pH can have an adverse effect on soil permeability and soil microflora [53]. Total solid (TS) levels were higher in both AU and BU, which can lead to high turbidity of the water bodies into which these effluents are discharged. In the present study, higher levels of total dissolved solids (TDS) indicated high salt content in the effluents analyzed. Paul et al. [51] also reported higher values of TDS (2264–7072 mg/L) in textile effluents. The higher values of TDS are due to the addition of different chemicals during pulping and bleaching processes, which can have detrimental effects on aquatic flora and fauna.

The degree of hardness becomes higher as the calcium and magnesium content increases and is related to the concentration of multivalent cations dissolved in the water. The change in alkalinity depends on carbonates and bicarbonates, which in turn depends upon the release of CO_2. The amount of total alkalinity in the effluents during the present study ranged from 356.667 to 656.667 mg/L. The hardness of water is mainly due to the presence of calcium and magnesium ions, and it is an important indicator of the toxic effect of poisonous elements [54].

Table 2. Physico-chemical characteristics (Mean ± S.E.) of collected samples (textile industrial effluents and soil) from Ludhiana, Punjab (India).

Parameter	AU	AT	BU	BT	BIS Limits [a]	Soil	Soil Limits [b]
pH	6.67 ± 0.05	7.49 ± 0.00 *	7.43 ± 0.02	8.90 ± 0.02 *	6.5–8.5	8.02 ± 0.01	6.5–8.5
EC (µS/cm)	1908.67 ± 4.67	628.67 ± 1.86 *	1858.33 ± 1.67	511.00 ± 2.00 *	-	442.5 ± 4.79	450
TDS (mg/L)	3366.33 ± 6.67	201.67 ± 1.67 *	3666.67 ± 13.33	1813.33 ± 35.28 *	500–2000	-	-
TS (mg/L)	3473.33 ± 6.67	786.67 ± 13.34 *	3893.33 ± 13.33	2000.00 ± 40.00 *	-	-	-
TSS (mg/L)	106.67 ± 6.67	585 ± 12.58 *	226.67 ± 13.33	186.67 ± 13.33	-	-	-
Alkalinity (mg/L)	656.67 ± 33.34	456.67 ± 33.34 *	490.00 ± 57.74	356.67 ± 33.33	200–600	-	-
Hardness (mg/L)	111.33 ± 6.67	151.33 ± 6.67 *	191.33 ± 6.67	104.67 ± 6.67 *	200–600	-	-
Calcium (mg/L)	33.93 ± 2.67	17.90 ± 2.67 *	60.65 ± 2.67	28.59 ± 2.67 *	75–200	120.24 (mg/kg) ± 0.00	0–3500 mg/kg
Magnesium (mg/L)	17.58 ± 4.40	70.33 ± 4.40 *	26.37 ± 7.61	21.98 ± 4.40 *	30–100	176.64 (mg/kg) ± 6.09	0–500 mg/kg
Sodium (mg/L)	333.63 ± 1.62	308.20 ± 1.25 *	141.08 ± 0.58	262.42 ± 1.04 *	-	343.08 (mg/kg) ± 3.02	0–300 mg/kg
Chloride (mg/L)	232.41 ± 4.73	142.47 ± 4.73 *	114.07 ± 4.73	66.74 ± 0.58 *	250–1000	-	-
Phosphate (mg/L)	1.58 ± 0.03	1.48 ± 0.02	2.08 ± 0.13	1.50 ± 0.03 *	-	-	-
Bulk density (g/cc)	-	-	-	-	-	1.08 ± 0.01	-
Sand (%)	-	-	-	-	-	33.49 ± 0.72	-
Silt (%)	-	-	-	-	-	26.05 ± 0.19	-
Clay (%)	-	-	-	-	-	40.45 ± 0.68	-
TOC (%)	-	-	-	-	-	2.22 ± 0.15	-

(AU: Untreated effluent of textile industry A; AT: Treated effluent of textile industry A; BU: Untreated effluent of textile industry B; BT: Treated effluent of textile industry B; TOC: Total organic carbon). [a] BIS [55]; [b] Awashthi [56], * Indicates statistically significant difference between values of parameters in untreated and treated effluents of the same industry. (Independent Student's *t*-test, $p \leq 0.05$).

3.2. Heavy Metal Estimation

3.2.1. Heavy Metal Contents in Industrial Effluents

The results of a metal analysis of industrial effluents are given in Table 3. The contents (mg/L) of Cd, Cr, Co, Cu, Pb, and Zn observed for untreated effluents of textile industry A were 0.004, 0.06, 1.72, 0.02, 0.13, and 0.09, respectively, while the values (mg/L) of these metals for treated effluents were below detection limit (BDL), 0.05, 1.33, 0.02, 0.11, and 0.02, respectively. The heavy metal contents observed for untreated effluent of textile industry B were in the order Co (1.69) > Cd (1.33) > Pb (0.14) > Zn (0.13) > Cr (0.06) > Cu (0.03), while in the case of treated effluent, the order was Co (1.42) > Pb (0.11) > Zn (0.07) > Cr (0.06) > Cu (0.01) > Cd (0.001). Among the heavy metals analyzed, the contents of Co were found to be above the standard limits for the discharge of effluents from textile industries.

Among all metals, Co content was observed to be high in untreated and treated effluents of both textile industries A and B as compared to the prescribed limits. All other tested heavy metals were found to be within the permissible level. Metal contamination in textile effluents was reported to occur because of the wide usage of chemicals, colorants, mordants, and other additives like caustic soda, sodium carbonate, etc. during the manufacturing processes [57]. Adinew [58] also reported that different heavy metals such as cobalt, copper, and chromium in textile effluents were present within the dye chromophores. The presence of heavy metals in effluents produces several adverse effects on living organisms [59]. Metals like chromium, zinc, iron, mercury, and lead were reported to pose environmental challenges [60]. In the present study, there was a significant difference ($p > 0.05$) in Co, Pb, and Zn content between untreated and treated effluents of both textile industries.

Table 3. Heavy metal contents (Mean ± S.E.) of collected samples (effluent and soil) from Ludhiana, Punjab (India).

Heavy Metal	Content of Heavy Metals (mg/L) of Effluent				Normal Acceptable Range (USEPA)		Content of Heavy Metals (mg/kg) in Soil	Indian Limits for Soil (mg/kg) [a]	European Union Standards (mg/kg) [b]
	AU	AT	BU	BT		FAO,1985			
Cadmium	0.004 ± 0.00	N.D.	0.002 ± 0.00	0.001 ± 0.00	2	0.01	1.33 ± 0.05	3–6	1
Chromium	0.06 ± 0.00	0.05 ± 0.00 *	0.06 ± 0.00	0.06 ± 0.00	2	0.10	16.43 ± 0.60	-	100
Cobalt	1.72 ± 0.00	1.33 ± 0.00 *	1.69 ± 0.00	1.42 ± 0.00 *	-	0.05	214.60 ± 0.42	-	50
Copper	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00 *	3	0.20	13.63 ± 1.88	135–270	100
Lead	0.13 ± 0.00	0.11 ± 0.00 *	0.14 ± 0.00	0.11 ± 0.00 *	0.1	5	57.33 ± 1.20	250–500	100
Zinc	0.09 ± 0.00	0.02 ± 0.00 *	0.13 ± 0.00	0.07 ± 0.00 *	5	2	92.52 ± 0.06	300–600	300

(AU: Untreated effluent of textile industry A; AT: Treated effluent of textile industry A; BU: Untreated effluent of textile industry B; BT: Treated effluent of textile industry B). * Indicates statistically significant difference between values of heavy metals in untreated and treated effluents of the same industry (Independent Student's *t*-test, $p \leq 0.05$). [a] Awashthi [56]; [b] European Union Standards (EU) [61].

3.2.2. Heavy Metal Contents in Soil

Table 3 shows the contents of various studied heavy metals in the soil of the agricultural field collected from the vicinity of textile industries. The contents (mg/kg) of Cd, Cr, Co, Cu, Pb, and Zn observed in samples were 1.33, 16.43, 214.60, 13.63, 57.33, and 92.52, respectively.

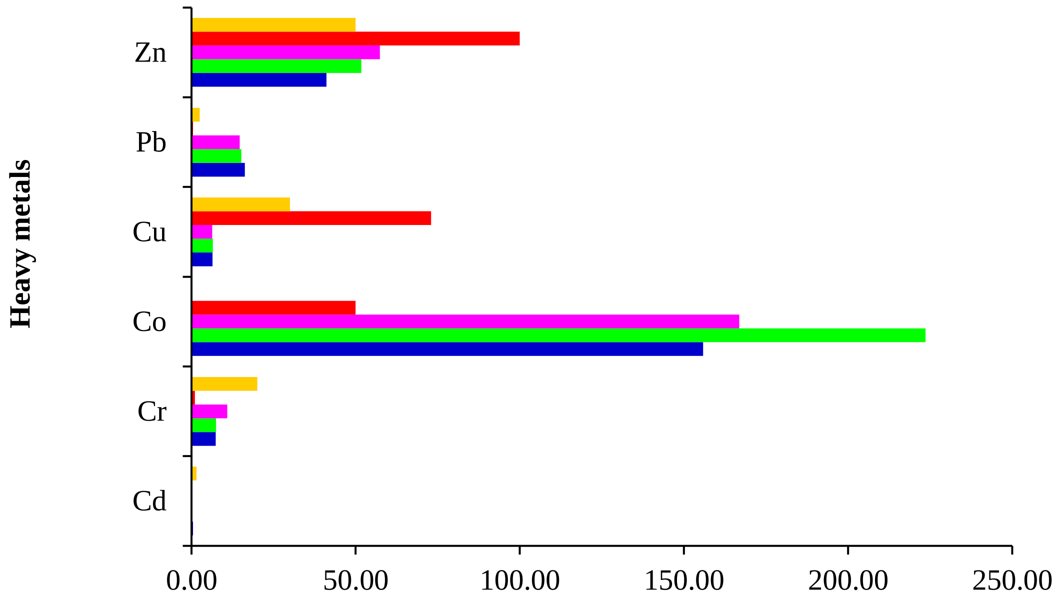
The chief sources of heavy metals in the roadside agricultural soils were documented to be the parent rock material, vehicular emissions, industrial activities, and agrochemicals like fertilizers and pesticides used for cultivation [18,62]. In the present study, soil samples were collected from agricultural fields in the vicinity of the textile industries. In the present study, three metals, that is, Cu, Cr, and Zn were observed to be low while Pb was higher in comparison to heavy metal contents reported from other parts of Punjab [63,64]. Also, Cd content was observed to be high in the present study in comparison to other parts of the world [65–70]. Cadmium is a toxic metal that causes serious health problems to humans, animals, and plants. Bhatti et al. [61] reported that the sources of the high levels of cadmium in the agricultural soils of Punjab were due to the usage of various agrochemicals like NPK (nitrogen, phosphate, potassium) fertilizers, pesticides, weedicides, etc. Industrial activities, lead mines, farmyard manure, and sewage sludge applications, etc. are reported to be the main sources of lead pollution in agriculture and plants [71]. Zinc pollution in roadside soils was caused by traffic-related activities such as vehicular emissions and weathering of crash barriers [18,72,73].

Among the different metals analyzed, the content of Co was observed to be maximum in the soil sample analyzed during the present study. Cobalt is documented to occur naturally in soils following two main pathways, that is, weathering of rocks comprising of minerals and breakdown of organic matter. The major mechanism involved in cobalt content in soil includes the anthropogenic usage of cobalt salts. Smaller amounts of cobalt can also enter the soil from the airborne transport of particulate emissions and application of sewage sludge onto fields. Heavy metal mobility in soil was reported to be inversely related to the strength of adsorption by soil constituents. However, the adsorption of cobalt to soils was reported to be rapid by Kim et al. [74]. Cobalt is reported to be one of the beneficial elements for the growth of higher plants although there is no report available regarding its direct role in plant metabolism [75]. However, some studies showed that cobalt was required for nitrogen fixation by bacteria in the root nodules of plants belonging to the leguminous family [76,77]. Cobalt has also been documented to be necessary for the processes of stem growth, elongating the coleoptiles, and expanding leaf discs. Moreover, cobalt reduced the peroxidase activity resulting in the breakdown of Indole acetic acid (IAA). Application of cobalt through seed treatments improved the germination of a seed, stand establishment, growth, yield, and quality [78]. Yet, a higher concentration of cobalt was found to be toxic, causing chlorosis and necrosis and inhibited root growth by retarding cell division, hindering the uptake and translocation of nutrients and water [78,79].

Copper and zinc are considered essential elements for plant nutrition, however, these can also cause toxic effects, if their concentrations exceed the required limits [80]. Plants mainly absorb zinc as a divalent cation, which acts either as a metal component or enzymes or as a functional, structural, or regulatory co-factor of many enzymes [81]. Despite being a non-essential element, cadmium can also get highly accumulated in plants as reported by Nadian [82]. Pb being a toxic element decreases the biomass growth and disrupts the total chlorophyll content of plants [83]. Naureen et al. [84] observed that the concentration of heavy metals in plants varied from species to species. Another study reported that the accumulation of selected metals varied greatly among plant species and uptake of an element by a plant was primarily dependent on the plant species and the soil characteristics [85]. Similar observations were also made by Rattan et al. [86]. On the other hand, Muchuweti et al. [87] reported that the excessive accumulation of heavy metals in soils was due to elevated levels of heavy metals in wastewater used for irrigation that led to increased uptake of metals in crops.

3.2.3. Heavy Metal Contents in Leaves of Plants

figfig:soilsystems-1285565-f001 shows the heavy metal contents in leaf samples of three plants *Cannabis sativa* L., *Chenopodium album* L., and *Ricinus communis* L. from the study area. The order of the heavy metals in the leaves of the three plants was observed to be Co > Zn > Pb > Cr > Cu > Cd. However, among three plant species, the order of heavy metal contents observed for Cd and Pb was *Cannabis sativa* L. > *Ricinus communis* L. > *Chenopodium album* L.; for Cr and Zn the order was *Chenopodium album* L. > *Ricinus communis* L. > *Cannabis sativa* L.; for Co the order was *Ricinus communis* L. > *Chenopodium album* L. > *Cannabis sativa* L.; and for Cu it was *Ricinus communis* L. > *Cannabis sativa* L. > *Chenopodium album* L.



	Cd	Cr	Co	Cu	Pb	Zn
Indian standards	1.50	20.00	0.00	30.00	2.50	50.00
FAO/WHO limits	0.20	1.00	50.00	73.00	0.43	100.00
Chenopodium album L	0.12	10.90	166.87	6.30	14.67	57.36
Ricinus communis L.	0.23	7.50	223.57	6.53	15.13	51.69
Cannabis sativa L.	0.41	7.33	155.80	6.43	16.20	41.14

Concentration (mg/kg)

Figure 1. Heavy metal contents in three plant species viz., *Cannabis sativa* L., *Ricinus communis* L., and *Chenopodium album* L. FAO/WHO, Adapted with permission from [56]. 2001, Indian standards: Awashthi.

In all the collected plant samples, the concentrations of different metals were observed to be above the European permissible limits like 50 mg/kg for cobalt, 0.2 mg/kg for cadmium, and 1 mg/kg for chromium. However, two metals and contents were determined to be lower than the Indian standards like Cd < 1.5 mg/kg and Cr < 20 mg/kg [88,89]. The concentration of copper in all three plant species was recorded to be less than both Indian standards of 30 mg/kg and European permissible limits of 73 mg/kg. Lead content was found to be higher than both Indian standards (2.5 mg/kg) and European permissible limits (0.43 mg/kg). The leaves of *Ricinus communis* L. and *Chenopodium album* L. had a higher zinc content than the Indian standards of 50 mg/kg and lower than that of European limits of 100 mg/kg. The content of zinc in *Cannabis sativa* L. was recorded to be less than both Indian standards and European permissible limits.

The variations of heavy metal contents in soil have a direct influence on the accumulation of heavy metals in plants. However, the heavy metal accumulation in plants also depends on the type of plant species, plant organelles, and traffic density. The plant species included in the present study were preferred because these were wildly grown along the boundaries of the agricultural field at the time of sampling. The contribution of human beings to metal concentrations in the terrestrial environment has arisen mainly from mining, smelting, and industrial activities [90].

3.3. Metal Bioaccumulation Factor (BAF)

BAF is one of the main indices that provide insight into the heavy metal uptake capacity of plant species. In the present study, BAF values were used to estimate and compare the extent of accumulation of various metals such as Cd, Co, Cr, Cu, Pb, and Zn in leaves of the three plants from the soil. Figure 2 presents the BAF values for different plant samples collected during the study. A BAF value above 1 was observed only for cobalt (1.042) in the leaves of *Ricinus communis* L, which indicates a high level of metal bioaccumulation in *Ricinus communis* L. The order of accumulation of heavy metals in the leaves of *Ricinus communis* L. was Co (1.04) > Zn (0.56) > Cu (0.48) > Cr (0.46) > Pb (0.26) > Cd (0.18); for *Chenopodium album* L. the order was Co (0.78) > Cr (0.66) > Zn (0.62) > Cu (0.46) > Pb (0.26) > Cd (0.09); and for *Cannabis sativa* L. it was Co (0.73) > Cu (0.47) > Cr (0.45) > Cd (0.31) > Pb (0.28).

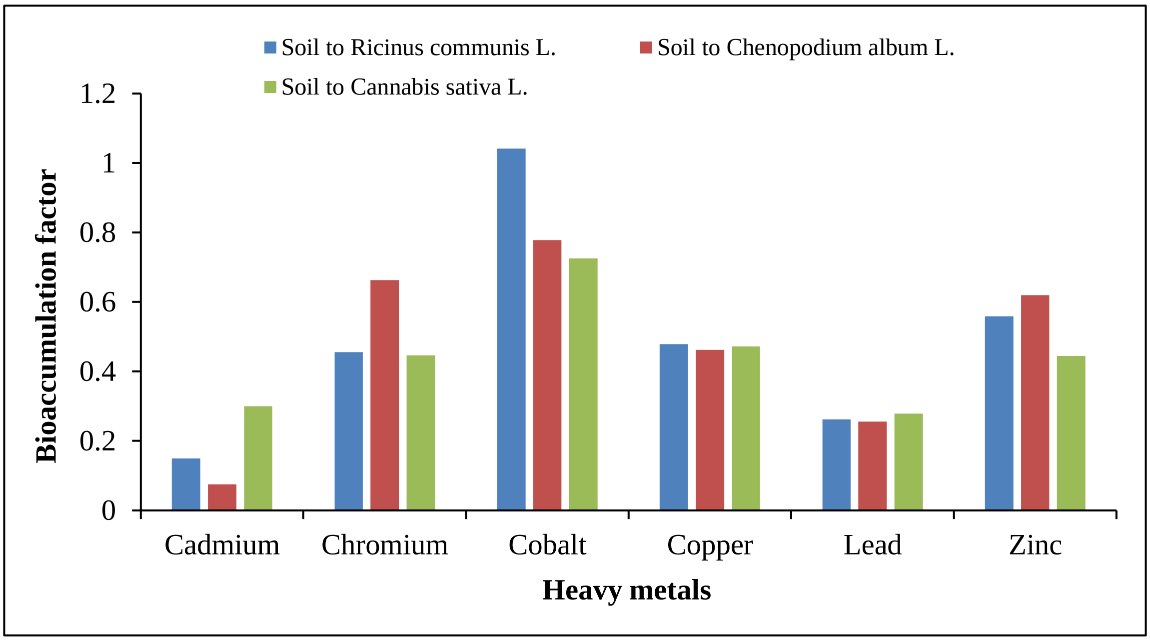


Figure 2. Bioaccumulation factor of Soil to plant.

The heavy metal accumulation examination provides very important information about the phytoremediation potential of the plant species. Transport and accumulation of heavy metals from soil to edible parts act as the major pathway for the entry of heavy metals into the food chain, which ultimately leads to various harmful effects [91]. The exposure of heavy metals to human beings leads to various health problems such as nervous system disorder, skin ailments, stomach problems, kidney damage, bone, and lung diseases [92–94]. The BAF of heavy metals depends upon the bioavailability of metals, which in turn depends upon the concentration of metal in soil, its chemical forms, the difference in uptake capability for different metals, and the growth rate of different plant species [53,95]. Different metals are accumulated in plants at variable rates depending on various factors such as physiology, requirements, and the metal uptake mechanism of plants, the physico-chemical characteristics of soil such as soil texture, soil pH and soil organic matter, as well as quantity of heavy metals present in the soil [94,96–98]. According to the guidelines given by Baker [99], BAF values greater than 1 indicated that the plant was an accumulator for the metal being analyzed and considered as harmful for plant health [53,100]. It is documented that *Ricinus communis* has good tolerance and phytoremediation potential for the removal of nickel (Ni) from contaminated land areas [101,102]. In the present study also, maximum BAF values for Co were observed in *Ricinus communis* L., which showed that this plant had higher metal bioaccumulation capacity than *Chenopodium album* L. and *Cannabis sativa* L. The mechanisms of cobalt accumulation are still not properly defined. However, there are some Cu accumulators which have the potential for Co accumulation as well due to similar mechanisms in the accumulation of different heavy metals [103]. Considering the Co toxicity on the targeted cellular system of plants that can hyperaccumulate, Co has been found to be evolved in regulating Fe homeostasis thus avoiding the accumulation of free ions that can induce oxidative stress [104]. Wong et al. [105] reported that heavy metals in carbonate-bounded form were more bioavailable than the presence of metal in any other fractions. Tamoutisidis et al. [106] revealed that heavy metals were transported passively from the root system to the shoot system through xylem vessels and were accumulated in the zones of high transpiration rates.

3.4. Genotoxicity of Industrial Effluent

The genotoxic potential of textile industrial effluents, before and after treatment was evaluated on the basis of percent aberrant cells. The results of genotoxic potential analysis of effluents and distilled water (negative control) using the *Allium cepa* test system are shown in Table 4. Among all physiological aberrations, delayed anaphases and stickiness were the most frequent type of aberrations while chromatin bridges dominated among clastogenic aberrations. Total chromosomal abnormalities in the meristematic cells of root tips of *Allium cepa* exposed to untreated effluents were significantly higher as compared to those exposed to treated effluents. The reduced mitotic index clearly indicates the cell division reduction in the root meristematic cells, which may be due to the collaborating effects of a complex mixture of cytotoxic chemicals like metals present in the textile industrial effluents. The total percentage aberration including both physiological (laggards, vagrants, stickiness, delayed anaphases, and c-mitosis) and clastogenic (chromatin bridges and chromosomal breaks) aberrations are shown in Figure 3. The total percent aberrant cells were observed to be 29.36% for AU, 27.48% for BU, 19.69% for AT, 16.52% for BT, and 3.84% for the negative control. The results obtained indicate the less toxic nature of the treated effluents of both textile industries (A and B) as compared to the untreated effluents, which can be due to the decrease in heavy metal contents.

Table 4. Genotoxic potential of industrial effluents collected from textile industries of Ludhiana (Punjab), India.

Parameter	NC	AU	AT	BU	BT
Average TDC	494	431	608	500	668
MI (%)	44.37 ± 1.01	13.83 ± 0.13 $^{\#}$	22.17 ± 0.40 $^{\#,*}$	17.46 ± 0.21 $^{\#}$	24.32 ± 0.26 $^{\#,*}$
PA (%)	3.43 ± 0.19	26.67 ± 0.30 $^{\#}$	17.50 ± 0.22 $^{\#,*}$	26.08 ± 0.48 $^{\#}$	15.13 ± 0.55 $^{\#,*}$
CA (%)	0.41 ± 0.01	2.70 ± 0.17 $^{\#}$	2.19 ± 0.15 $^{\#}$	1.40 ± 0.20 $^{\#}$	1.40 ± 0.06 $^{\#}$
TA (%)	3.84 ± 0.19	29.37 ± 0.40 $^{\#}$	19.69 ± 0.36 $^{\#,*}$	27.48 ± 0.44 $^{\#}$	16.52 ± 0.59 $^{\#,*}$

(NC: Negative Control; (AU: Untreated effluent of textile industry A; AT: Treated effluent of textile industry A; BU: Untreated effluent of textile industry B; BT: Treated effluent of textile industry B); TDC: Total Dividing Cells; MI: Mitotic Index; PA: Physiological Aberration; CA: Clastogenic Aberration; TA: Total Aberration). * Indicates statistically significant difference between values of genotoxicity parameters (MI, PA, CA, and TA) for untreated and treated effluents of same industry (Independent Student's *t*-test, $p \leq 0.05$). # Indicates statistically significant difference between values of genotoxicity parameters (MI, PA, CA, and TA) for effluents and negative control (Chi square test, $p \leq 0.05$).

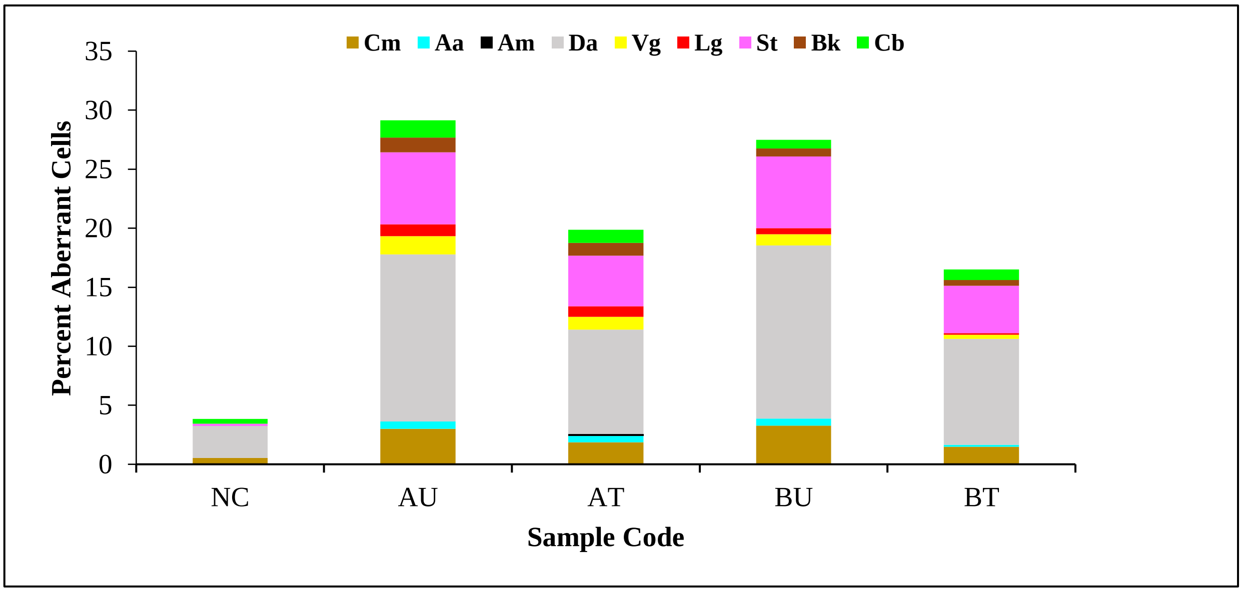


Figure 3. Induction of physiological and clastogenic chromosomal aberrations in root tip cells of *Allium cepa* under exposure to industrial effluents and distilled water (Negative Control). Physiological aberrations (Cm: C-mitosis; Da: Delayed anaphase; Lg: Laggards; St: Stickiness; Vg: Vagrants; Aa: Abnormal anaphases; Am: Abnormal metaphases); Clastogenic aberrations (Cb: Chromatin bridges; Bk: Chromosomal breaks), Sample codes (AU: Untreated effluent of textile industry A; AT: Treated effluent of textile industry A; BU: Untreated effluent of textile industry B; BT: Treated effluent of textile industry B); NC: Negative control.

The results observed in the present study indicate the mitogenic, as well as the clastogenic effects of the textile effluents, which were evident from the low value of the mitotic index (MI) and higher values of the chromosomal aberrations assay when compared to the results obtained from treatment with negative control (distilled water). The statistical analysis (Chi square test) also revealed that there is a significant difference between the values of the genotoxicity parameters viz., physiological aberration (PA), clastogenic aberration (CA), and total aberration (TA) along with mitotic index (MI) for effluents of both textile industries (A and B) and negative control at $p \leq 0.5$). Chromosomal aberration is an important indicator for assessing the genotoxicity of textile effluents [107]. The *Allium cepa* root chromosomal aberration assay has been widely used for cytotoxic as well as genotoxic mitotic studies [41,108–110]. The reduction in the values of mitotic index, in the present study, indicated the cytotoxic effects, whereas the induction of chromosomal and nuclear abnormalities showed genotoxic effects. Both cytotoxic and genotoxic effects were endorsed by various environmental pollutants [44].

The higher genotoxic response of root tip cells under exposure to untreated effluent of textile industry A as compared to untreated effluent of B industry can be attributed to the presence of high content of Cd, Co, Na, and Cl. Some authors have reported the cytotoxic and genotoxic effects using the *Allium cepa* test system following exposure to heavy metals [111,112]. Cd has been shown to reduce the mitotic index (MI) and enhance the induction of chromosomal aberrations, as well as micronuclei, in various studies [113–116]. Grover and Kaur [117] studied the genotoxic potential of textile and paper mill effluents and sewage water following the *Allium cepa* chromosomal aberration assay and reported that the industrial effluents induced the formation of micronuclei and chromosomal abnormalities in the root tip cells of *Allium cepa*. Genotoxicity of both untreated and treated textile industrial effluents was evaluated using the *Allium cepa* test system by Vijayalakshmidevi and Muthukumar, [118] and they observed a reduction in the mitotic index, as well as induction of various types of chromosomal aberrations in the root tips exposed to effluent. It is possible that some chemicals in the complex chemical mixtures could have stimulatory effects on the mitotic process while some others might have mito-depressive effects [43]. Similar results were also reported for genotoxicity of textile industrial effluents using the *Allium cepa* test system by other authors [42,108,119,120] and they demonstrated the induction of chromosomal abnormalities and decrease in the mitotic index in root tips cells treated with the effluent. Therefore, mitotic responses observed in this study could be due to the overall collaborative effects such as additive, antagonistic, and synergistic of the complex chemical mixtures in the effluents on the root meristematic cells.

3.5. Pollution Assessment

The Igeo, *CF*, Cd_{eg}, mCd_{eg}, PI, PLI, ER_i and RI of the agricultural soil in the present study were calculated based on the heavy metals content in the studied soil sample. Table 5 indicates that the *CF* value of heavy metals in studied area was ranked as: Co (21.46) > Cd (13.57) > Pb (2.87) > Zn (1.30) > Cu (0.55) > Cr (0.47) and the Igeo value ranked as Co (3.84) > Cd (3.18) > Pb (0.93). Pollution levels of contamination factor (*CF*) were classified as: low contamination (*CF* < 1), moderate contamination (*CF* value in the range of 1–3), considerable contamination (*CF* value in the range of 3–6), and very high contamination (*CF* > 6) by Taylor and Mclennan [121] and Hakanson [122]. The *CF* value indicated that the soil is extremely polluted by Cd and Co, moderately polluted by Pb and Zn whereas unpolluted by Cu and Cr. The result of Igeo showed that soil is heavily contaminated by Cd and Co whereas uncontaminated by Cr, Cu, and Zn on the basis of the classification given by Muller [123] and Taylor and Mclennan [121]. Considering the Cd_{eg} values > 32, PI values > 3, and PLI > 3, the studied soil was found to be extremely polluted with heavy metals whereas mCd_{eg} (6.70) value in the present study indicated that soil has a high degree of contamination. ER_i values for Cr, Cu, Pb, and Zn were observed to be below 40, indicating low potential ecological risk from these metals whereas Cd showed considerable potential ecological risk and Co exhibited very high potential ecological risk. In the present work, ER_i values showed that Co is the major pollutant in the area which indicates that agricultural management is a probable cause of heavy metals accretion. The potential ecological risk index (RI) demonstrated that the study area had considerable ecological risk considering the RI value in the range of 300–600.

Table 5. Metal pollution indices for collected soil samples from Ludhiana, Punjab (India).

Metal	Igeo	CF	Cd_{eg}	mCd_{eg}	PI	PLI	ER_i	RI
Cd	3.18	13.57	40.22	6.70	15.90	26.41	407.14	533.74
Cr	−1.68	0.47					0.94	
Co	3.84	21.46					107.3	
Cu	−1.46	0.55					2.73	
Pb	0.93	2.87					14.33	
Zn	−0.203	1.30					1.30	

Igeo: geoaccumulation index; CF: contamination factor; Cd_{eg}: degree of contamination; mCd_{eg}: modified degree of contamination; PI: Numerow's pollution index; PLI: pollution load index; ER_i: potential ecological risk factor; RI: potential ecological risk index.

3.6. Human Health Risk Assessment

The non-carcinogenic, hazard quotient (*HQ*), and hazard index (*HI*) of analyzed heavy metals (Cd, Cr, Co, Cu, Pb, and Zn) through three exposure pathways, that is, ingestion, dermal contact, and inhalation for adults and children were calculated and results were shown in Table 6. The values of $HQ_{ingestion}$, HQ_{dermal}, and $HQ_{inhalation}$ for all studied heavy metals were found to be lower than 1 for both adults and children and thus indicated that there is no obvious risk to the population. The total carcinogenic risks (TCR) were calculated only for Cr as cancer slope factors (*SF*) for all three exposure pathways (ingestion, dermal contact, and inhalation) are not available for other heavy metals. The total carcinogenic risk value was found to be in the range of the permissible limit of 1×10^{-6} to 1×10^{-4}, as provided by USEPA [28]. The results of cancer risk were shown in Figure 4.

Table 6. Exposure values for non-carcinogenic risks for adults and children from different exposure pathways in the study area.

Receptor	Exposure Pathway	Cd	Cr	Co	Cu	Pb	Zn
Adult	ADI ingestion	1.9×10^{-6}	2.347×10^{-5}	3.066×10^{-4}	1.947×10^{-5}	8.19×10^{-5}	1.322×10^{-4}
	ADI dermal	5.786×10^{-8}	7.147×10^{-7}	9.335×10^{-6}	5.929×10^{-7}	2.494×10^{-6}	4.0246×10^{-6}
	AID inhalation	1.788×10^{-10}	2.209×10^{-9}	2.885×10^{-8}	1.833×10^{-9}	7.708×10^{-9}	1.244×10^{-8}
	Total	1.958×10^{-6}	2.42×10^{-5}	3.159×10^{-4}	2.01×10^{-5}	8.440×10^{-5}	1.36×10^{-4}
	HQ ingestion	1.9×10^{-3}	7.824×10^{-3}	1.533×10^{-2}	4.868×10^{-4}	5.85×10^{-2}	4.406×10^{-4}
	HQ dermal	5.786×10^{-3}	2.382×10^{-4}	5.834×10^{-4}	4.941×10^{-5}	4.759×10^{-3}	6.708×10^{-5}
	HQ inhalation	1.788×10^{-7}	7.724×10^{-5}	5.053×10^{-3}	4.582×10^{-8}	2.190×10^{-6}	4.147×10^{-8}
	HI	7.686×10^{-3}	8.139×10^{-3}	2.097×10^{-2}	5.362×10^{-4}	6.326×10^{-2}	5.077×10^{-4}
Children	ADI ingestion	1.33×10^{-5}	1.643×10^{-4}	2.146×10^{-3}	1.363×10^{-4}	5.733×10^{-4}	9.252×10^{-4}
	ADI dermal	2.128×10^{-8}	2.629×10^{-7}	3.434×10^{-6}	2.181×10^{-7}	9.173×10^{-7}	1.480×10^{-6}
	ADI inhalation	3.731×10^{-10}	4.609×10^{-9}	6.020×10^{-8}	3.823×10^{-9}	1.608×10^{-8}	2.595×10^{-8}
	Total	1.332×10^{-5}	1.64567×10^{-4}	2.149×10^{-3}	1.365×10^{-4}	5.742×10^{-4}	9.267×10^{-4}
	HQ ingestion	1.33×10^{-2}	5.477×10^{-2}	0.1073	3.408×10^{-3}	0.410	3.084×10^{-3}
	HQ dermal	2.128×10^{-3}	8.763×10^{-5}	2.146×10^{-4}	1.817×10^{-5}	1.751×10^{-3}	2.467×10^{-5}
	HQ inhalation	3.731×10^{-7}	1.611×10^{-4}	1.054×10^{-2}	9.559×10^{-8}	4.569×10^{-6}	8.651×10^{-8}
	HI	1.543×10^{-2}	5.502×10^{-2}	0.118	3.426×10^{-3}	0.411	3.109×10^{-4}

(ADI: Average daily intake; HQ: Hazard quotient; HI: Hazard index).

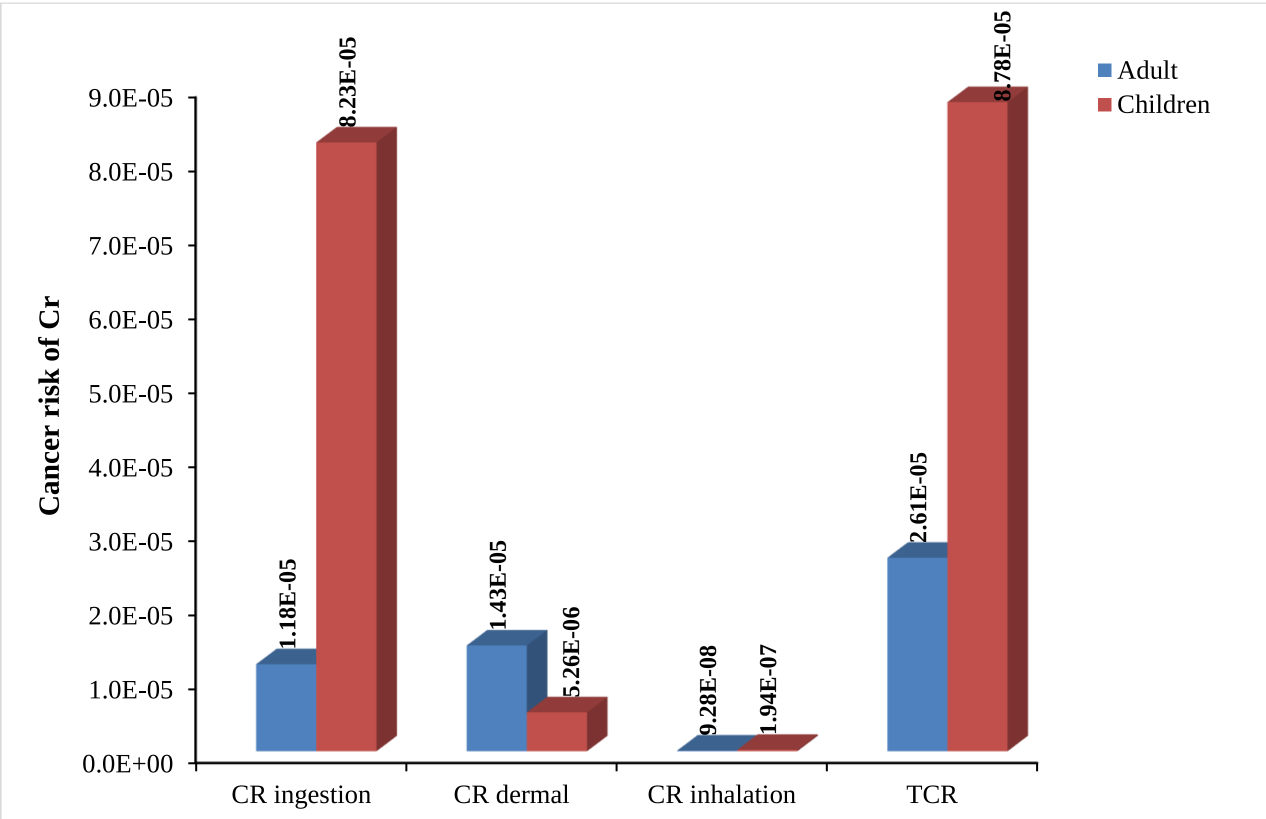


Figure 4. Distribution of carcinogenic risk of chromium (Cr) for adults and children in the study area. (CR; carcinogenic risk; TCR: total carcinogenic risk).

4. Conclusions

The present study pertained to exploring the potential ecological risks of heavy metals of textile effluents in soil samples in the vicinity of textile industries Ludhiana, Punjab (India). The metal bioaccumulation potential of some plant species grown in its environs was also explored. The Co content in untreated and treated effluent samples indicated the possibility of accumulation of cobalt in agricultural soil samples and plant samples in the vicinity of textile industries, which is a serious matter of concern. The genotoxicity assay showed that treated as well as untreated effluents of both industries induced chromosomal aberrations and the percent aberrations in treated samples were significantly lower than untreated samples. The heavy metal bioaccumulation factor analysis showed that phytoremediation using wildly grown plants like *Ricinus communis* L., *Chenopodium album* L. and *Cannabis sativa* L., can be one of the environmentally friendly techniques for cleaning contaminated soil environs. Furthermore, Igeo and *CF* revealed that heavy metals showed no contamination to extreme contamination in the studied soil whereas Cd_{eg}, PI, and PLI indicated extreme pollution. The results of ER_i studies indicated that Co is the prime metal responsible for ecological threats in the study area. It is also emphasized that bioanalytical tools such as the *Allium cepa* root chromosomal aberration assay should be incorporated along with chemical analysis for evaluating the efficacy of industrial effluent treatment plants so as to indicate the harmful consequences in the biological systems.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/soilsystems5040063/soilsystems5040063/s1, Table S1: Descriptions of the soil contamination indices used in the study, Table S2. Summary of reference doses (*RfD*) and slope factors (*SF*) of heavy metals.

Author Contributions: J.K.: She has carried out all experimental and has drafted/written the manuscript; S.S.B.: Helped in statistical analysis of data; S.A.B.: review and editing the manuscript; A.K.N.: Contributed for assessment of health risk; V.K.: Co-supervisor of the first author and helped in reviewing the whole manuscript; J.K.K.: Supervisor of the first author and helped in the drafting of manuscript/guidance for result compilation and finalizing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University Potential for Excellence (UPE) as the first author is recipient of fellowship under this program.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors are thankful to the University Grants Commission (UGC), New Delhi for providing financial assistance through various schemes like University Potential for Excellence (UPE), Centre with Potential for Excellence in Particular Area (CPEPA), Department of Science and Technology—Promotion of University Research and Scientific Excellence (DST-PURSE), Departmental Research Support-Special Assistance Program (DRS-SAP). Thanks are due to the Head of the Department of Botanical and Environmental Sciences for providing laboratory facilities and the Central Facility, Emerging Life Sciences, Guru Nanak Dev University, Amritsar for providing sophisticated instrumentation to carry out the present work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tabrez, S.; Ahmad, M. Oxidative stress-mediated genotoxicity of wastewaters collected from two different stations in northern India. *Mutat. Res.* **2011**, *726*, 15–20. [CrossRef] [PubMed]
2. Dey, S.; Islam, A. A Review on Textile Wastewater Characterization in Bangladesh. *Resour. Environ.* **2015**, *5*, 15–44.
3. Brevik, E.C.; Slaughter, L.; Singh, B.R.; Steffan, J.J.; Collier, D.; Barnhart, P.; Pereira, P. Soil and human health: Current status and future needs. *Air Soil Water Res.* **2020**, *13*, 1–23. [CrossRef]
4. Mandour, R.A.; Azab, Y.A. The Prospective Toxic Effects of Some Heavy Metals Overload in Surface Drinking Water of Dakahlia Governorate, Egypt. *J. Occup. Environ. Med.* **2011**, *2*, 245–253.
5. Huang, D.; Liu, X.; Jiang, S.; Wang, H.; Wang, J.; Zhang, Y. Current state and future perspectives of sewer networks in urban China. *Front. Environ. Sci. Eng.* **2018**, *12*, 2. [CrossRef]
6. Singh, S.; Kumar, M. Heavy metal load of soil, water and vegetables in periurban Delhi. *Environ. Monit. Assess.* **2006**, *120*, 79–91. [CrossRef]

7. Sharma, R.K.; Agarwal, M.; Marshall, F.M. Heavy metals contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicol. Environ. Saf.* **2007**, *66*, 258–266. [CrossRef]
8. Zehra, S.; Arshad, M.; Mahmood, T.; Waheed, A. Assessment of heavy metal accumulation and their translocation in plant species. *Afr. J. Biotechnol.* **2009**, *8*, 2802–2810.
9. Doherty, V.F.; Sogbanmu, T.O.; Kanife, U.C.; Wright, O. Heavy metals in vegetables collected from selected farm and market sites in Lagos, Nigeria. *Glob. Adv. Res. J. GJAR* **2012**, *1*, 137–142.
10. Egito, L.C.M.; Medeiros, M.D.G.; Medeiros, S.R.B.D.; Agnez-Lima, L.F. Cytotoxic and genotoxic potential of surface water from the Pitimbu river, Northeastern/RN Brazil. *Gen. Mol. Biol.* **2007**, *30*, 435–441. [CrossRef]
11. Alam, Z.M.; Ahmad, S.; Malik, A.; Ahmad, M. Genotoxic and mutagenic potential of agricultural soil irrigated with tannery effluents at Jajmau (Kanpur), India. *Arch. Environ. Contam. Toxicol.* **2009**, *57*, 463–476. [CrossRef]
12. Alam, Z.M.; Ahmad, S.; Malik, A.; Ahmad, M. Mutagenicity and genotoxicity of tannery effluents used for irrigation at Kanpur, India. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1620–1628. [CrossRef]
13. Magdaleno, A.; Puig, A.; de Cabo, L.; Salinas, C.; Arreghini, S.; Korol, S.; Bevilacqua, S.; Liopez, L.; Moretton, J. Water pollution in an urban Argentine river. *Bull. Environ. Contam. Toxicol.* **2001**, *67*, 408–415. [CrossRef] [PubMed]
14. Ohe, T.; Watanabe, T.; Wakabayashi, K. Mutagens in surface waters: A review. *Mutat. Res.* **2004**, *567*, 109–149. [CrossRef]
15. Siddiqui, A.H.; Ahmad, M. The *Salmonella* mutagenicity of industrial, surface and ground water sample of Aligarh region of India. *Mutat. Res.* **2003**, *541*, 21–29. [CrossRef]
16. Fatoki, O.S. Trace Zinc and Copper Concentrations in Roadside Vegetation and Surface Soils: A Measurement of Local Atmospheric Pollution in Alice, South Africa. *Int. J. Environ. Stud.* **2000**, *57*, 501–513. [CrossRef]
17. Keshavarzi, A.; Kumar, V.; Ertunc, G.; Brevik, E.C. Ecological risk assessment and source apportionment of heavy metals contamination: An appraisal based on the Tellus soil survey. *Environ. Geochem. Health* **2021**, *43*, 2121–2142. [CrossRef] [PubMed]
18. Bhatti, S.S.; Bhatt, S.A.; Kumar, V.; Kaur, M.; Sambyal, V.; Singh, J.; Vig, A.P.; Nagpal, A.K. Ecological risk assessment of metals in roadside agricultural soils: A modified approach. *Hum. Ecol. Risk Assess.* **2018**, *24*, 186–201. [CrossRef]
19. Kachenko, A.G.; Singh, B. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water Air Soil Poll.* **2006**, *169*, 101–123. [CrossRef]
20. Atafar, Z.; Mesdaghinia, A.; Nouri, J.; Homaee, M.; Yunesian, M.; Ahmadimoghaddam, M.; Mahvi, A.H. Effect of fertilizer applications on soil heavy metal concentration. *Environ. Monit. Assess.* **2010**, *160*, 83–89. [CrossRef]
21. Cui, Y.J.; Zhu, Y.G.; Zhai, R.H.; Chen, D.Y.; Huang, Y.Z.; Qiu, Y.; Liang, J.Z. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environ. Int.* **2004**, *30*, 785–791. [CrossRef]
22. Lee, C.S.; Li, X.D.; Shi, W.Z.; Cheung, S.C.; Thornton, L. Metal contamination in urban, suburban and country park soils of Hong Kong: A study based on GIS and multivariate statistics. *Sci. Total Environ.* **2006**, *356*, 45–61. [CrossRef]
23. Tepanosyan, G.; Sahakyan, L.; Belyaeva, O.; Maghakyan, N.; Saghatelyan, A. Human health risk assessment and riskiest heavy metal origin identification in urban soils of Yerevan, Armenia. *Chemosphere* **2017**, *184*, 1230–1240. [CrossRef] [PubMed]
24. Adimalla, N.; Wang, H. Distribution, contamination, and health risk assessment of heavy metals in surface soils from northern Telangana, India. *Arab. J. Geosci.* **2018**, *11*, 684. [CrossRef]
25. Chen, H.Y.; Teng, Y.G.; Lu, S.J.; Wang, Y.; Wang, J. Contamination features and health risk of soil heavy metals in China. *Sci. Total Environ.* **2015**, *512*, 143–153. [CrossRef]
26. Eziz, M.; Mohammad, A.; Mamut, A.; Hini, G. A human health risk assessment of heavy metals in agricultural soils of Yanqi Basin, Silk Road Economic Belt, China. *Hum. Ecol. Risk Assess.* **2018**, *24*, 1352–1366. [CrossRef]
27. Adimalla, N. Heavy metals pollution assessment and its associated human health risk evaluation of urban soils from Indian cities: A review. *Environ. Geochem. Health* **2020**, *42*, 173–190. [CrossRef]
28. US Environmental Protection Agency. *Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites*; OSWER 9355; Office of Emergency and Remedial Response, US Environmental Protection Agency: Washington, DC, USA, 2002; pp. 4–24.
29. Eaton, A.D.; Clesceri, L.S.; Rice, E.W.; Greenberg, A.E. *Standard Methods for The Examination of Water and Wastewater*, Centennial ed.; American Public Health Association, American Water Works Association, Water Environment Federation: Washington, DC, USA, 2005.
30. Kumar, V.; Bhatti, S.S.; Nagpal, A.K. Seasonal analysis of physico-chemical parameters of agricultural soil samples collected from banks of rivers Beas and Sutlej, Punjab, India. *J. Chem. Pharm. Res.* **2016**, *8*, 439–449.
31. Trivedi, R.K.; Goel, P.K.; Trisal, C.L. (Eds.) Aquatic ecosystem. In *Practical Methods in Ecology and Environmental Sciences*; Enviro Media Pub: Bhopal, India, 1987; pp. 57–113.
32. Nelson, D.W.; Sommer, L.E. Total Carbon, Organic Carbon and Organic Matter. Methods of Soil Analysis, Part 2. In *Chemical and Microbiological Properties*, 2nd ed.; ASA-SSSA: Madison, WI, USA, 1982; pp. 579–595.
33. Jacob, H.; Clarke, G. Part 4. Physical method. In *Methods of Soil Analysis*; Soil Science Society of America: Madison, WI, USA, 2002; p. 1692.
34. ISO 11277. *Soil Quality-Determination of Particle Size Distribution in Mineral Soil Material-Method by Sieving and Sedimentation*; ISO: Geneva, Switzerland, 2009.
35. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association: Washington, DC, USA, 1998.

36. Sharma, D.; Katnoria, J.K.; Vig, A.P. Chemical changes of spinach waste during composting and vermicomposting. *Afr. J. Biotechnol.* **2011**, *10*, 3124–3127.
37. Ehi-Eromosole, C.O.; Adaramodu, A.A.; Anake, W.U.; Ajanaku, C.O.; Edobor-Osoh, A. Comparison of three methods of digestion for trace metal analysis in suface dust collected from an Ewaste recycling site. *Nat. Sci.* **2012**, *10*, 1–6.
38. Allen, S.E.; Grimshaw, H.M.; Rowland, A.P. Chemical Analysis. In *Methods in Plant Ecology*; Moore, P.D., Chapman, S.B., Eds.; Blackwell Scientific Publications: Oxford, UK; London, UK, 1986; pp. 285–344.
39. Huang, M.; Zhou, S.; Sun, B.; Zhao, Q. Heavy metals in wheat grain: Assessment of potential health risk for inhabitants in Kunshun, China. *Sci. Total Environ.* **2008**, *405*, 54–61. [CrossRef] [PubMed]
40. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals-concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [CrossRef] [PubMed]
41. Katnoria, J.K.; Arora, S.; Bhardwaj, R.; Nagpal, A.K. Evaluation of genotoxic potential of industrial waste contaminated soil extracts of Amritsar, India. *J. Environ. Biol.* **2011**, *32*, 363–367. [PubMed]
42. Pathiratne, A.; Hemachandra., C.K.; De Silva, N. Efficacy of *Allium cepa* test system for screening cytotoxicity and genotoxicity of industrial effluents originated from different industrial activities. *Environ. Monit. Assess.* **2015**, *187*, 1–12. [CrossRef]
43. Hemachandra, C.K.; Pathiratne, A. Combination of physico-chemical analysis, *Allium cepa* test system and *Oreochromis niloticus* erythrocyte based comet assay/nuclear abnormalities tests for cyto-genotoxicty assessments of treated effluents discharged from textile industries. *Ecotoxicol. Environ. Saf.* **2016**, *131*, 54–64. [CrossRef]
44. Leme, D.M.; Marin-Morales, M.A. *Allium cepa* test in environmental monitoring: A review on its application. *Mutat. Res.* **2009**, *682*, 71–81. [CrossRef]
45. US Environmental Protection Agency. Risk Assessment Guidance for Superfund. In *Human Health Evaluation Manual, Part A*; EPA/540/1–89/002; Office of Emergency and Remedial Response, US Environmental Protection Agency: Washington, DC, USA, 1989; Volume 1.
46. US Environmental Protection Agency. Guidelines for carcinogen risk assessment, EPA/630/P-03/001F. In *Risk Assessment Forum*; US Environmental Protection Agency: Washington, DC, USA, 2005.
47. US Environmental Protection Agency. *Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (part F, Supplemental Guidance for Inhalation Risk Assessment)*; OSWER 9285; Office of Superfund Remediation and Technology Innovation, US Environmental Protection Agency: Washington, DC, USA, 2009; pp. 7–82.
48. US Environmental Protection Agency. *Exposure Factors Handbook*, 2011 ed.; EPA/600/R-09/052F; National Center for Environmental Assessment: Washington, DC, USA, 2011.
49. US Environmental Protection Agency. *Exposure Factors Handbook*; Volume 1: General Factors; US Environmental Protection Agency, Office of Research and Development: Washington, DC, USA, 1997.
50. Li, Z.; Ma, Z.; van der Kuijp, T.J.; Yuan, Z.; Huang, L. A review of soil heavy metal pollution from mines in China: Pollution and health risk assessment. *Sci. Total Environ.* **2014**, *468*, 843–853. [CrossRef]
51. Paul, S.A.; Chavan, S.K.; Khambe, S.D. Studies on characterization of textile industrial waste water in Solapur city. *Int. J. Chem. Sci.* **2012**, *10*, 632–642.
52. Ramamurthy, N.; Balasaraswathy, S.; Sivasakthivelan, P. Biodegradation and Physico-chemical changes of textile effluent by various fungal species. *Rom. J. Biophys.* **2011**, *21*, 113–123.
53. Singh, A.; Sharma, R.K.; Agrawal, M.; Marshall, F.M. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food Chem. Toxicol.* **2010**, *48*, 611–619. [CrossRef]
54. Vyas, P.B. An aerobic treatment for Dudh Sagar dairy, Mahesana. *J. Ind. Pollut. Control.* **2011**, *27*, 29–32.
55. Bureau of Indian Standards. *Indian Standard Drinking Water Specification*; Bureau of Indian Standards: New Delhi, India, 2012; p. 10500.
56. Awashthi, S.K. (Ed.) *Central and State Rules as Amended for 1999: Prevention of Food Adulteration Act No. 37 of 1954*; Ashoka Law House: New Delhi, India, 2000.
57. Yaseen, D.A.; Scholz, M. Textile dye wastewater characteristics and constituents of synthetic effluents: A critical review. *Int. J. Environ. Sci. Technol.* **2018**, *16*, 1193–1226. [CrossRef]
58. Adinew, B. Textile effluent treatment and decolorization techniques—A review. *Chem. Bulg. J. Sci. Educ.* **2012**, *21*, 434–456.
59. Chukwu, L.O. Physico-chemical characterization of pollutant load of treated industrial effluents in Lagos metropolis, Nigeria. *J. Indus. Poll. Control* **2006**, *22*, 17–22.
60. Hussein, F.H. Chemical properties of treated textile dyeing wastewater. *Asian J. Chem.* **2013**, *25*, 9393–9400. [CrossRef]
61. European Union. Heavy Metals in Wastes, European Commission on Environment 2000. Available online: http://ec.europa.eu/environment/waste/studies/pdf/heavy_metalsreport.pdf (accessed on 21 July 2014).
62. Zhao, Z.; Hazelton, P. Evaluation of accumulation and concentration of heavy metals in different urban roadside soil types in Miranda Park, Sydney. *J. Soils Sediment.* **2016**, *16*, 2548–2556. [CrossRef]
63. Bhatti, S.S.; Sambyal, V.; Nagpal, A.K. Heavy metals bioaccumulation in Berseem (*Trifoliumalexandrium*) cultivated in areas under intensive agriculture, Punjab, India. *SpringerPlus* **2016**, *5*, 1–11. [CrossRef]
64. Bhatti, S.S.; Sambyal, V.; Singh, J.; Nagpal, A.K. Analysis of soil characteristics of different land uses and metal bioaccumulation in wheat grown around rivers: Possible human health risk assessment. *Environ. Dev. Sustain.* **2017**, *19*, 571–588. [CrossRef]

65. Aschale, M.; Sileshi, Y.; Kelly-Quinn, M.; Hailu, D. Pollution Assessment of Toxic and Potentially Toxic Elements in Agricultural Soils of the City Addis Ababa, Ethiopia. *Bull. Environ. Contam. Toxicol.* **2017**, *98*, 234–243. [CrossRef]
66. Chabukdhara, M.; Munjal, A.; Nema, A.K.; Gupta, S.K.; Kaushal, R.K. Heavy metal contamination in vegetables grown around peri-urban and urban-industrial clusters in Ghaziabad, India. *Hum. Ecol. Risk Assess.* **2016**, *22*, 736–752. [CrossRef]
67. Tian, K.; Huang, B.; Xing, Z.; Hu, W. Geochemical baseline establishment and ecological risk evaluation of heavy metals in greenhouse soils from Dongtoi, China. *Ecol. Indic.* **2017**, *72*, 510–520. [CrossRef]
68. Guan, Z.H.; Lil, X.G.; Wang, L. Heavy metal enrichment in roadside soils in the eastern Tibetan Plateau. *Environ. Sci. Pollut. Res.* **2018**, *25*, 7625–7637. [CrossRef] [PubMed]
69. Krailertrattanachai, N.; Ketrot, D.; Wisawapipat, W. The Distribution of Trace Metals in Roadside Agricultural Soils, Thailand. *Int. J. Environ. Res. Public Health* **2019**, *16*, 714. [CrossRef] [PubMed]
70. Szwalec, A.; Mundała, P.; Kędzior, R.; Pawlik, J. Monitoring and assessment of cadmium, lead, zinc and copper concentrations in arable roadside soils in terms of different traffic conditions. *Environ. Monit. Assess.* **2020**, *192*, 155. [CrossRef] [PubMed]
71. Khan, M.A.; Ahmad, I.; Rahman, I. Effect of Environmental Pollution on Heavy Metals Content of *Withamnia somnifera*. *J. Chin. Chem. Soc.* **2007**, *54*, 339–343. [CrossRef]
72. Ahmed, F.; Fakhruddin, A.N.M.; Imam, M.D.T.; Khan, N.; Khan, T.A.; Rahman, M.M.; Abdullah, A.T.M. Spatial distribution and source identification of heavy metal pollution in roadside surface soil: A study of Dhaka Aricha highway, Bangladesh. *Ecol. Process.* **2016**, *5*, 1–16. [CrossRef]
73. Aryal, R.; Beecham, S.; Sarkar, B.; Chong, M.N.; Kinsela, A.; Kandasamy, J.; Vigneswaran, S. Readily wash-off road dust and associated heavy metals on motorways. *Water Air Soil Pollut.* **2017**, *228*, 1–12. [CrossRef]
74. Kim, J.H.; Gibb, H.J.; Howe, P.D. Cobalt and Inorganic Cobalt Compounds. In *Concise International Chemical Assessment Document, No. 69*; World Health Organization: Geneva, Switzerland, 2006; pp. 1–82.
75. Gad, N. Role and importance of cobalt nutrition on groundnut (*Arachis hypogaea*) production. *World Appl. Sci. J.* **2012**, *20*, 359–367.
76. Witte, C.P.; Tiller, S.A.; Taylor, M.A.; Davies, H.V. Addition of Nickel to Murashiga and Skoog medium in plant tissue culture activates urease and may reduce metabolic stress. *Plant Cell Tiss. Org. Cult.* **2002**, *86*, 103–104. [CrossRef]
77. Nagpal, N.K. *Water Quality Guidelines for Cobalt*; Ministry of Water, Land and Air Protection, Water Protection Section, Water, Air and Climate Change Branch: Victoria, TX, USA, 2004.
78. Caselles, J.M.; Pérez-Espinosa, A.; Pérez Murcia, M.D.; Moral, R.; Gomez, I. Effect of Increased Cobalt Treatments on Cobalt Concentration and Growth of Tomato Plants. *J. Plant Nutr.* **1997**, *20*, 805–811. [CrossRef]
79. Jayakumar, K.; Jaleel, C.A. Uptake and accumulation of cobalt in plants: A study based on exogenous cobalt in soybean. *Bot. Res. Int.* **2009**, *2*, 310–314.
80. Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K.; Sutton, D.J. Heavy Metal Toxicity nad the Environment. *Mol. Clin. Environ. Toxicol. Exp. Suppl.* **2012**, *101*, 133–164. [CrossRef]
81. Alloway, B.J. *Heavy Metals in Soils*; Blackie Academic and Professional: London, UK, 1990; pp. 1–330.
82. Nadian, H. Cd and Mn uptake and bioaccumulation in *Trifolium alexandrinum* L.: Interaction with mycorrhizal colonization. In Proceedings of the Fourth International Iran and Russia Conference, Shahrekord, Iran, 8–10 September 2004; pp. 595–601.
83. McDermott, S.; Wu, J.; Cai, B.; Lawson, A.; Marjorie Aelion, C. Probability of intellectual disability is associated with soil concentrations of arsenic and lead. *Chemosphere* **2011**, *84*, 31–38. [CrossRef] [PubMed]
84. Naureen, A.; Irshad, M.; Hussain, F.; Mahmood, Q. Comparing heavy metals accumulation potential in natural vegetation and soil adjoining wastewater canal. *J. Chem. Soc. Pak.* **2011**, *33*, 661–665.
85. Chunilall, V.; Kindness, A.; Johnalagada, S.B. Heavy metal uptake by two edible Amaranthus herbs grown on soils contaminated with lead, mercury, cadmium and nickel. *J. Environ. Sci. Health* **2005**, *40*, 375–385. [CrossRef] [PubMed]
86. Rattan, R.K.; Datta, S.P.; Chhonkar, P.K.; Suribabu, K.; Singh, A.K. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—A case study. *Agric. Ecosyst. Environ.* **2005**, *109*, 310–322. [CrossRef]
87. Muchuweti, M.; Birkett, J.W.; Chinyanga, E.; Zvauya, R.; Scrimshaw, M.D.; Lester, J.N. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. *Agric. Ecosyst. Environ.* **2006**, *112*, 41–48. [CrossRef]
88. Lokeshwari, H.; Chandrappa, G. Impact of heavy metal contamination of Bellandur Lake on soil and cultivated vegetation. *Curr. Sci.* **2006**, *91*, 622–627.
89. Zango, M.S.; Anim-Gyampo, M.; Ampadu, B. Health risks of heavy metals in selected food crops cultivated in small-scale gold-mining areas in Wassa-Amenfi-West district of Ghana. *J. Nat. Sci.* **2013**, *3*, 96–105.
90. Collins, R.N.; Kinsela, A. The aqueous phase speciation and chemistry of cobalt in terrestrial environments. *Chemosphere* **2010**, *79*, 763–771. [CrossRef] [PubMed]
91. Nasar, H.M.; Sultan, S.; Gomes, R.; Noor, S. Heavy metal pollution of soil and vegetable grown near roadside at Gazipur. *Bangladesh J. Agri. Res.* **2012**, *37*, 9–17. [CrossRef]
92. Duruibe, J.O.; Ogwuegbu, M.D.C.; Egwurugwu, J.N. Heavy metal pollution and human biotoxic effects. *Int. J. Phys. Sci.* **2007**, *2*, 112–118.
93. Kim, H.; Song, B.; Kim, H.; Park, J. Distribution of trace metals at two abandoned mine sites in Korea and arsenic-associated health risk for the residents. *Toxicol. Environ. Health Sci.* **2009**, *1*, 83–90. [CrossRef]

94. Khan, A.; Khan, S.; Khan, M.A.; Qamar, Z.; Waqas, M. The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: A review. *Environ. Sci. Pollut. Res.* **2015**, *22*, 13772–13799. [CrossRef]
95. Bi, X.; Feng, X.; Yang, Y.; Li, X.; Shin, G.P.Y.; Li, F. Allocation and source attribution of lead and cadmium in maize (*Zea mays* L.) impacted by smelting emissions. *Environ. Pollut.* **2009**, *157*, 834–839. [CrossRef]
96. Ahmad, J.U.; Goni, M.A. Heavy metal contamination in water, soil, and vegetables of the industrial areas in Dhaka, Bangladesh. *Environ. Monit. Assess.* **2010**, *166*, 347–357. [CrossRef]
97. Ding, C.; Zhang, T.; Wang, X.; Zhou, F.; Yang, Y.; Yin, Y. Effects of soil type and genotype on lead concentration in rootstalk vegetables and the selection of cultivars for food safety. *J. Environ. Manag.* **2013**, *122*, 8–14. [CrossRef]
98. Hu, J.; Wu, F.; Wu, S.; Sun, X.; Lin, X.; Wong, M.H. Phytoavailability and phytovariety codetermine the bioaccumulation risk of heavy metal from soils, focusing on Cd-contaminated vegetable farms around the Pearl River Delta, China. *Ecotoxicol. Environ. Saf.* **2013**, *91*, 18–24. [CrossRef] [PubMed]
99. Baker, A.J. Accumulators and excluders-strategies in the response of plants to heavy metals. *J. Plant Nutr.* **1981**, *3*, 643–654. [CrossRef]
100. Bashir, M.; Khalid, S.; Rashid, U.; Adrees, M.; Ibrahim, M.; Islam, M.S. Assessment of selected heavy metals uptake from soil by vegetation of two areas of district Attock, Pakistan. *Asian J. Chem.* **2014**, *26*, 1063–1068. [CrossRef]
101. Ma, Y.; Rajkumar, M.; Freitas, H. Isolation and characterization of Ni mobilizing PGPB from serpentine soils and their potential in promoting plant growth and Ni accumulation by *Brassica* spp. *Chemosphere* **2009**, *75*, 719–725. [CrossRef]
102. Adhikari, T.; Kumar, A. Phytoaccumulation and tolerance of *Ricinus communis* L. to nickel. *Int. J. Phytoremediation* **2012**, *14*, 481–492. [CrossRef]
103. Faucon, M.P.; Shutcha, M.N.; Meerts, P. Revisiting copper and cobalt concentrations in supposed hyperaccumulators from SC Africa: Influence of washing and metal concentrations in soil. *Plant Soil* **2007**, *301*, 29–36. [CrossRef]
104. Oven, M.; Grill, E.; Golan-Goldhirsh, A.; Kutchan, T.M.; Zenk, M.H. Increase of free cysteine and citric acid in plant cells exposed to cobalt ions. *Phytochemistry* **2002**, *60*, 467–474. [CrossRef]
105. Wong, S.C.; Li, X.D.; Zhang, G.; Qi, S.H.; Min, Y.S. Heavy metals in agricultural soils of the Pearl River Delta, South China. *Environ. Pollut.* **2002**, *119*, 33–44. [CrossRef]
106. Tamoutsidis, E.; Lazaridou, M.; Papadopoulos, I.; Spanos, T.; Papathanasiou, F.; Tamoutsidou, M.; Mitlianga, P.; Vasiliou, G. The effect of treated urban wastewater on soil properties, plant tissue composition and biomass productivity in berseem clover and corn. *J. Food Agric. Environ.* **2009**, *7*, 782–786.
107. Jadhav, S.; Phugare, S.; Patil, P.S.; Jadhav, J.P. Biochemical degradation pathway of textile dye Remazol red and subsequent toxicological evaluation by cytotoxicity, genotoxicity and oxidative stress studies. *Int. Biodeterior. Biodegrad.* **2011**, *65*, 733–743. [CrossRef]
108. Carita, R.; Marin-Morales, M.A. Induction of chromosome aberrations in the *Allium cepa* test system caused by exposure of seeds to industrial effluents in contaminated with azo dyes. *Chemosphere* **2008**, *72*, 722–725. [CrossRef] [PubMed]
109. Panneerselman, N.; Palanikumar, L.; Gopinathan, S. Chromosomal aberration by Glycidol in *Allium cepa* L. root meristem cell. *Int. J. Pharm. Sci. Res.* **2012**, *3*, 300–304.
110. Kaur, J.; Kaur, V.; Pakade, Y.B.; Katnoria, J.K. A study on water quality monitoring of Buddha Nullah, Ludhiana, Punjab (India). *Environ. Geochem. Health* **2020**, *43*, 2699–2722. [CrossRef] [PubMed]
111. Barbosa, J.S.; Cabral, T.M.; Ferreira, D.N.; Agnez-Lima, L.F.; Batistuzzo de Medeiros, S.R. Genotoxicity assessment in aquatic environment impacted by the presence of heavy metals. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 320–325. [CrossRef]
112. Hemachandra, C.K.; Pathiratne, A. Assessing toxicity of copper, cadmium and chromium levels relevant to discharge limits of industrial effluents into inland surface waters using common onion, *Allium cepa* bioassay. *Bull. Environ. Contam. Toxicol.* **2015**, *94*, 199–203. [CrossRef]
113. Zhang, Y.X.; Yang, X.L. The toxic effects of cadmium on cell division and chromosomal morphology of *Hordeum vulgare*. *Mutat. Res.* **1994**, *312*, 121–126. [CrossRef]
114. Zhang, Y.; Xiao, H. Antagonistic effect of calcium, zinc and selenium against cadmium induced chromosomal aberrations and micro-nuclei in root cells of *Hordeum vulgare*. *Mutat. Res.* **1998**, *420*, 1–6. [CrossRef]
115. Fojtova, M.; Kovarik, A. Genotoxic effect of cadmium is associated with apoptotic changes in tobacco cells. *Plant Cell Environ.* **2000**, *23*, 531–537. [CrossRef]
116. Unyayar, S.; Celik, A.; Cekic, F.O.; Gozel, A. Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. *Mutagenesis* **2006**, *21*, 77–81. [CrossRef] [PubMed]
117. Grover, I.S.; Kaur, S. Genotoxicity of wastewater samples from sewage and industrial effluent detected by the *Allium* root anaphase aberration and micronucleus assays. *Mutat. Res. Fund. Mol. Mech. Mut.* **1999**, *426*, 183–188. [CrossRef]
118. Vijayalakshmidevi, S.R.; Muthukumar, K. Improved biodegradation of textile dye effluent by coculture. *Ecotoxicol. Environ. Saf.* **2015**, *114*, 23–30. [CrossRef]
119. Sudhakar, R.; Gowda, K.N.; Venu, G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytologia* **2001**, *66*, 235–239. [CrossRef]
120. Khan, S.; Anas, M.; Malik, A. Mutagenicity and genotoxicity evaluation of textile industry wastewater using bacterial and plant bioassays. *Toxicol. Rep.* **2019**, *6*, 193–201. [CrossRef]
121. Taylor, S.R.; Mclennan, S.M. The geochemical evolution of the continental crust. *Rev. Geophys.* **1995**, *33*, 241–265. [CrossRef]

122. Hakanson, L. An ecological risk index for aquatic pollution control. A sedimentological approach. *Water Res.* **1980**, *14*, 975–1001. [CrossRef]
123. Muller, G. Index of geoaccumulation in sediments of the Rhine River. *J. Geol.* **1969**, *2*, 108–118.