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QUANTIFICATION OF HEAVY METAL CONTAMINATION IN SOIL AND PLANTS NEAR A LEATHER TANNING INDUSTRIAL AREA USING LIBS AND TXRF**

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The present work reports the quantitative analysis of chromium along with other constituents in the soil of the Jajmau industrial area, Kanpur, India using laser-induced breakdown spectroscopy (LIBS) and total reflection X-ray fluorescence (TXRF). High concentrations of chromium have also been demonstrated in wheat and mustard plants in this study.

Keywords: *laser-induced breakdown spectroscopy, total reflection X-ray fluorescence, chromium, tanning, soil contamination.*

КОЛИЧЕСТВЕННАЯ ОЦЕНКА ЗАГРЯЗНЕНИЯ ТЯЖЕЛЫМИ МЕТАЛЛАМИ ПОЧВЫ И РАСТЕНИЙ В ЗОНЕ КОЖЕВЕННОГО ПРОИЗВОДСТВА С ИСПОЛЬЗОВАНИЕМ ЛАЗЕРНО-ИСКРОВОЙ СПЕКТРОСКОПИИ И РЕНТГЕНОВСКОЙ ФЛУОРЕСЦЕНЦИИ

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Проведен количественный анализ содержания хрома наряду с другими составляющими в почве Джаймауской промышленной зоны (Канпур, Индия) методами спектроскопии лазерно-индуцированной плазмы (LIBS) и рентгеновской флуоресценции с полным внешним отражением (TXRF). Высокая концентрация хрома наблюдается также в растениях пшеницы и горчицы.

Ключевые слова: *спектроскопия лазерно-индуцированной плазмы, рентгеновская флуоресценция с полным внешним отражением, хром, дубление, загрязнение почвы.*

Introduction. The discharge of industrial effluents near lands being used for agricultural activities and the use of industrial wastewater for agricultural practices are one of the many reasons for the increase in heavy metal pollution of soil and groundwater. Reliable measurement techniques for evaluating heavy metal over a wide range of matrices should be developed for modeling the fate of industrial pollution on agricultural soils. Additionally, it has been found that crops grown in such fields are contaminated with pollutants. Among heavy metals, chromium is widely used in the leather industry. Soils of urban or agricultural areas located near industrial zones are the major absorbers of contamination released from industrial effluents [1, 2]. In soil, some metals like chromium persist for longer times because of their fairly immobile nature, while other metals migrate either to groundwater aquifers or to plants due to their mobility. Chromium is common-

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ly used in leather tanning. The effluents of these industries, if not treated properly, lead to an increase of the Cr content in soil, crop, and groundwater of nearby areas, which in turn can find their way into the human body through respiration or through ingestion of water and food. Through the food chain, chromium is present in the environment in several oxidation states. The most common forms are trivalent and hexavalent, Cr(III) and Cr(VI). Chromium(III) is an essential nutrient required by the human body to promote the action of insulin in body tissues for the utilization of sugar, protein, and fat by the body [3]. High levels of Cr(VI), however, can cause irritation to the nose, leading to symptoms such as runny nose, sneezing, itching, nosebleeds, ulcers, and holes in the nasal septum. Long-term exposure to chromium has been associated with lung cancer in workers exposed to high (100–1000 times higher than natural environments) levels of chromium in air [4]. Another route of entry of chromium into the human body is via consumption of plants having high Cr levels by virtue of being cultivated on heavy metal contaminated soils. Thus, the monitoring of Cr contaminated lands is of particular importance to identify the trends of pollution and for planning remedial activities.

Monitoring toxic heavy metals in contaminated lands requires the development of such analytical detection techniques that can be used to quantify matrices of different complexity, including groundwaters and soils of different genesis. LIBS is a powerful method in which elemental profiling of various samples [5–8] can be performed. In the LIBS technique, interaction of the intense laser radiation and sample leads to plasma formation. As the plasma cools down, it gives emissions corresponding to elements present in the sample. The objective of this work was not only to develop suitable techniques for monitoring toxic metals in the soil, but also to highlight the uptake of these metals by edible plants being grown in this industrial area.

Jajmau is a leather tanning industrial area located near Kanpur City, Uttar Pradesh, India. Previous studies have highlighted the presence of high amounts of Cr in this area [9, 10]. These reports have also suggested that plants take up the metals present in these contaminated soils, including toxic metals like Cr [11–15]. Therefore, it is of utmost importance to characterize the plant samples with respect to heavy metal concentrations. In the present work, we have estimated the concentrations of toxic heavy metal Cr along with other constituent elements present in the soil and plant samples collected from nearby industrial areas of Jajmau, Kanpur, India, using laser-induced breakdown spectroscopy (LIBS) and total reflection X-ray fluorescence (TXRF).

Experimental. Soil samples, along with wheat and mustard plant samples. were collected from the fields near the Jajmau industrial area of Kanpur, Uttar Pradesh, India (Table 1).

TABLE 1. Sample Specifications

Sample name	Sample details
Soil 1 and 2	Collected from 0.5–1.0 km distance from industries at two different locations.
Soil 3 and 4	Collected from 3 km distance from industries at two different locations.
Soil 5	Collected from the canal through which the wastewater flows.
Soil 6	Collected from 6–7 km away from industries.
Seed 1 and Seed 2	Wheat seed samples growing on the field from which Soil 1 and Soil 2 were collected.
Seed 3	Mustard seed samples growing on the field from which Soil 3 was collected.
Leaf 1 and Leaf 2	Leaves of wheat plants growing on the field from which Soil 1 was collected.
Leaf 3	Leaves of wheat plants growing on the field from which Soil 2 was collected.

LIBS. The soil samples were dried in an oven at 80°C overnight. They were then ground and sieved using a nylon cloth (mesh size 120 μm) to obtain samples of a uniform particle size. Sample pellets were obtained by subjecting 100 mg of powdered soil samples to a pressure of ~5 tons using a hydraulic press and KBr die.

LIBS spectra of the soil sample pellets were recorded using optimized experimental conditions such as the lens-to-sample distance, the laser energy per pulse, and the repetition rate for this study. The LIBS experimental setup used for the analysis of the soil samples is described in detail elsewhere [8, 9]. The components of the LIBS setup include a Nd:YAG laser operating at a wavelength of 532 nm and capable of delivering a maximum energy of 425 mJ over a pulse duration of 4 ns (Continuum Surelite III-10, USA), a sample stage, an optical fiber cable having collecting lens inclined at an angle of ~45° relative to the direction of the incident laser beam, and a spectrometer with a CCD detector (Ocean Optics, LIBS 2000+) having a fixed gate delay of 1.5 μs. A 15 cm focal length lens was used to focus the laser beam on the surface of the soil pellets.

TXRF. The leaves and seeds of the plants were separated and washed thoroughly with Millipore water in order to remove surface contamination. The samples were then dried in an oven at 60°C overnight. Further they were crushed using a mortar and pestle. The ground samples were sieved using nylon cloth with a mesh size of 120 μm to obtain a uniform particle size. For the TXRF analysis, accurately weighed amounts of the dried leaf and seed samples were digested using 4 mL of concentrated ultrapure nitric acid and 2 mL of 30% high purity H_2O_2 using a hot plate. Two reference materials, namely, wheat seeds (NCS ZC730096, GSB-2) and tea leaves (GSB-7), were also digested along with the experimental samples to validate the TXRF results.

Since the bio-accumulation of different elements in plants depends on the extent of elements that are extractable in water, experiments were also performed to estimate the amounts of water extractable elements present in the soil samples. This was performed by shaking 200 mg of the soil samples (“Soil 1”, “Soil 2”, and “Soil 3’) in 1 mL of Millipore water using a mechanical shaker overnight (~16 h). The solutions were then filtered using Whatmann filter paper No. 42, and 20 μL of each solution was analyzed by TXRF.

An Ital Structures TXRF spectrometer (TX-2000) with a molybdenum target tube was used for the TXRF measurements. The K_α molybdenum radiation obtained from the tube was operated at 40 kV and 20 mA and monochromatized by a W-C multilayer used for the excitation of the elements in the sample. The characteristic X-rays emitted from the samples were detected with the help of a Si(Li) detector having a resolution of 139 at 5.9 keV. The TXRF spectra were recorded using a TXRFACQ 32 program and were processed using the EDXRF program provided with the instrument.

Results and discussion. *LIBS analysis.* A typical LIBS spectrum from the collected soil sample in different spectral ranges is shown in Fig. 1. Identification of elements present in the sample was performed using atomic spectroscopic NIST data [16]. Several elements such as Ca, Mg, Na, Fe, Si, Al, Mn, K, and Zn were identified by the presence of their prominent spectral lines. The presence of Cr in the soil samples was indicated by the presence of the persistent spectral lines at 357.8, 359.3, 360.5, 425.4, 427.4, 428.9, 206.1,

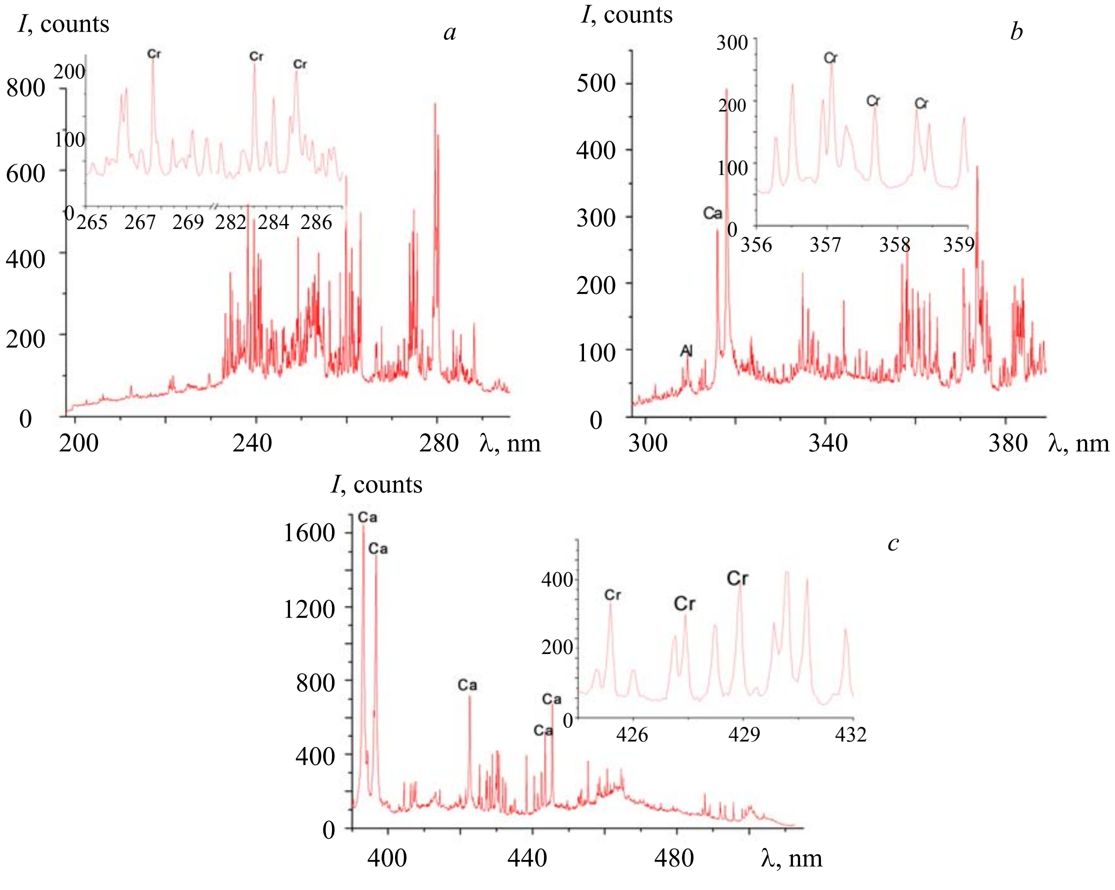

Fig. 1. LIBS spectrum of the soil sample in the spectral range of 200–300 (a), 300–400 (b), and 400–500 nm (c).

267.7, 283.5, 284.3, 284.9, and 285.5 nm. Chromium was quantified using a calibration curve method, for which Cr standard soils were prepared by spiking Cr free soil with a multi-elemental standard (M/s Merck India Ltd.). For this purpose, Cr concentrations (mg/kg) were used: 100, 200, 300, 400, 500, 1000, 1500, and 2000 ppm. The spectral intensity of Cr at wavelengths 357.8, 425.4, 267.7, and 206.1 nm was chosen to obtain the calibration curve.

The spectral response of the Cr emission lines at 357.8 and 425.4 nm (Fig. 2a,b) was found to be linear at lower concentrations (up to 1000 mg/kg of Cr), while a concentration increase beyond 1000 mg/kg led to a loss of linearity. This is due to the effects of self-absorption at higher concentrations (insets in Fig. 2a,b). Therefore, the calibration curves generated using the emission lines of 357.8 and 425.4 nm were used for the quantification at lower Cr concentrations. The calibration curves obtained using the emission lines 267.7 and 206.1 nm had linear responses up to 2000 mg/kg (Fig. 2c,d). The limit of detection (LOD) using the calibration curves for 267.7 and 206.1 nm was 12 and 15 ppm, respectively. Thus, for the quantification of Cr in unknown samples, the calibration curves of the emission lines 267.7 and 206.1 nm were used as they demonstrated better linearity as well as good detection limits.

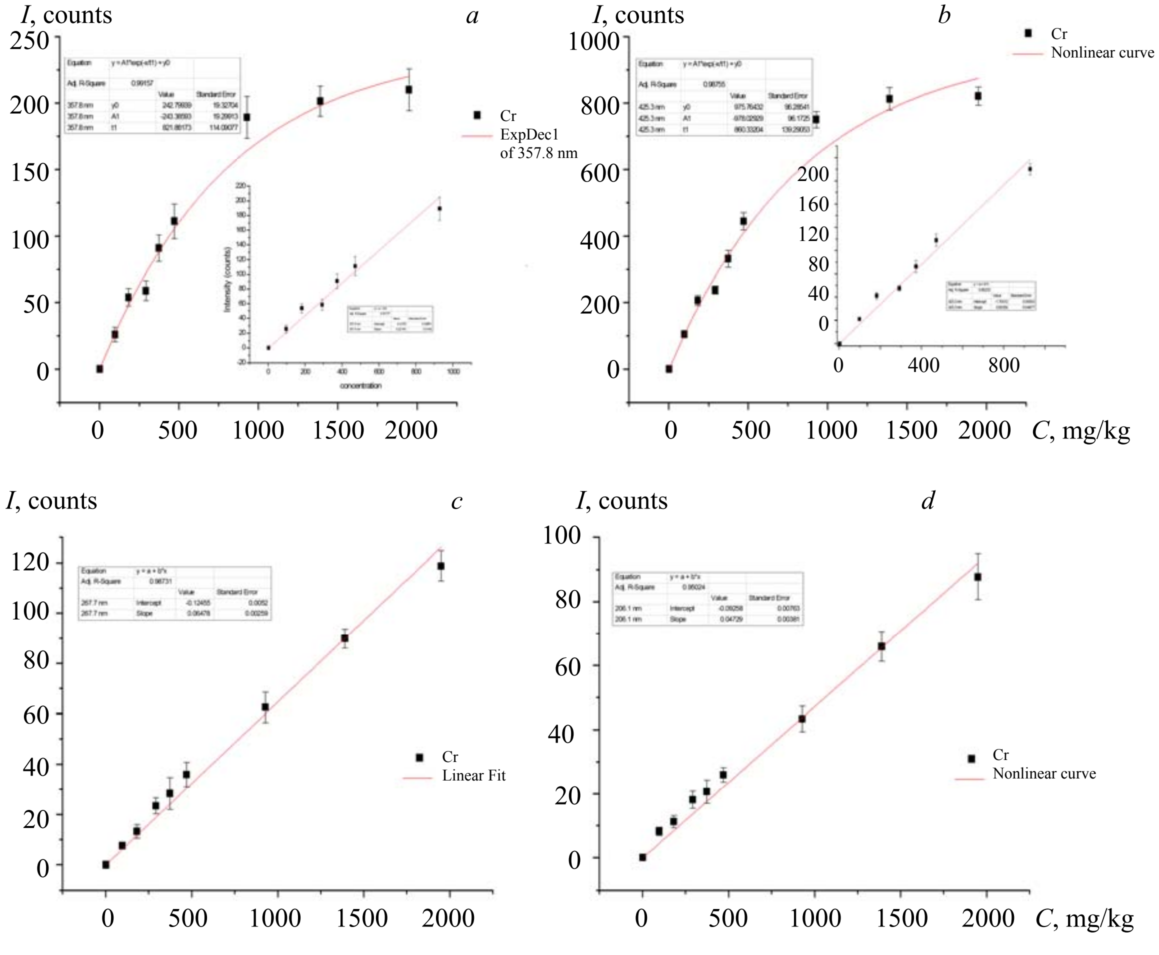

Fig. 2. Calibration curve for the spectral lines 357.8 (a), 425.4 (b), 267.7 (c), and 206.1 nm (d).

The concentrations of Cr estimated in the soil samples are shown in Table 2. The soil samples Soil 1, Soil 2, and Soil 3 collected from the fields near (within 0.5–3.0 km range) the industrial area shows higher amounts of Cr. The soil sample Soil 4 was collected from the same region as Soil 3 but shows higher Cr concentration. This may be attributed to the fact that this field was very dry and there were no crops at the time of the sample collection, leading to evaporation of the standing water in the land. The concentration of Cr in the soil sample collected from the canal through which the wastewater flows (Soil 5) was very high (>2000 mg/kg).

TABLE 2. Concentrations (mg/kg) of Cr in the Analyzed Soil Sample using LIBS

Sample	At 267.7 nm	At 206.1 nm
Soil 1	1090	901
Soil 2	1324	1180
Soil 3	185	185
Soil 4	754	563
Soil 5	>2000	>2000
Soil 6	41	38

The spectral intensities of the emission lines at 357.8, 425.4, 267.7, and 206.1 nm in the sample Soil 5 are much higher than for the highest concentration used in the calibration standard (2000 mg/kg) prepared for the calibration curve, and thus the quantification of Cr in this sample could not be carried out using the present calibration curve method. The soil sample Soil 6 collected from a larger distance (~6–7 km away from the industrial area) was found to have lower amounts of Cr. Therefore, the Cr contamination decreases as the distance increases due to Cr sedimentation in the canal over the larger distance. In our earlier study [17], we also observed that the amount of Cr adsorbed onto the particulate matter is higher than that dissolved in the water, and hence the sedimentation is the major cause for the decrease in Cr concentration with distance. Our results show that the concentrations of Cr in the agricultural fields near the leather tanning industrial area are at a dangerous level, in contrast to previous studies in which the average Cr content of the soil of different locations of Jajmau has been estimated as 195–435 mg/kg [18]. The previous study by our research group has also highlighted high Cr concentrations in the industrial effluents of Jajmau being discharged into the Ganga river [19]. In addition, previous studies have also quantified Cr and other heavy metals in the industrial sludge of Jajmau using LIBS and XRF to estimate its concentration in the solid waste [2, 17].

TXRF analysis. A relative sensitivity method was used for the quantification of different elements present in the plant samples. An internal standard (absent in the sample) was chosen, and the sensitivity factor for the characteristic lines of different elements (intensity/concentration) was used. The relative sensitivity values of different elements in the GSB-7 (NCS ZC73014) tea leaf standard relative to known concentrations of the internal standard gallium (Ga) were determined and were used to evaluate the concentrations of various elements in the samples. For the quantification of the elements, 20 μL of the digested sample was mixed with 20 μL of Ga (50 μg/mL), and 20 μL from this mixture was deposited on the quartz support and the TXRF spectrum was recorded for 1000 seconds in duplicate. The certified reference material NCSZC70312 (GSB-5) was also analyzed by the same procedure to validate the TXRF results.

The concentration of Cr in the soil extract was determined using Ga as an internal standard. The concentration of Cr in the soil extract obtained from the sample Soil 1 was estimated as 106 mg/kg. This represents the amount of Cr that was extracted from the soil sample, and using this information, the extent of leaching from the soils was evaluated as ~0.5% per hour.

A typical TXRF spectrum of the wheat leaf sample is shown in Fig. 3. The estimated concentrations of the different elements of the leaf samples by TXRF are shown in Table 3. In addition to Cr, concentration of Cu, Fe, K, and Ni were also determined by TXRF. The Cr concentration in the wheat seeds was found to be less than that of the mustard seeds (obtained from Soil 3). Mustard seed samples collected from SOIL 3 (having less Cr content) were found to accumulate higher amounts of Cr, similar to previous studies [20]. Thus, mustard holds the potential to be used for bio-remediation of heavy metal contaminated soils.

TABLE 3. Concentrations (mg/kg) of Elements Present in the Plant Sample using TXRF

Element	Seed 1	Seed 2	Seed 3	Leaf 1	Leaf 2	Leaf 3
Ca	166±11	294±8	2310±10	8559±28	6897±32	10397±34
Cr	3.33±0.47	6.79±0.52	8.21±0.49	85.7±1.6	97.6±2.2	82± 1
Cu	2.4±0.2	2.2±0.2	2.9±0.2	3.3 ± 0.3	12.4±0.4	13.4±0.3
Fe	40.6±0.3	36.5±0.3	54.83±0.2	604±2	691±3	979±4
Mn	10.8±0.9	13.6±0.8	27.7±0.9	115.6±1.7	102±2	112±1
Zn	55.2±2.3	36.77±2.2	78.92±2.5	26.8±0.4	56.1±0.6	37.1±0.4

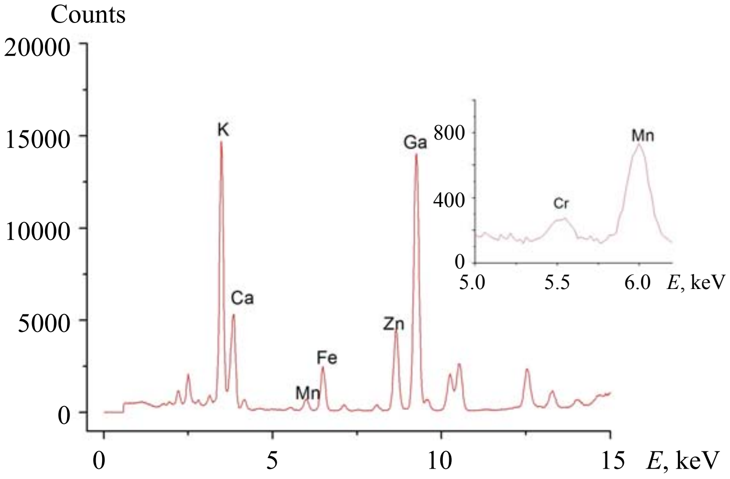

Fig. 3. TXRF spectrum of the leaf 1 sample.

The presence of Cr in the leaves of and seeds of wheat and mustard indicates that the crops growing in nearby fields are contaminated with heavy metals to a significant extent. The results of this field study show that the use of industrial effluents for irrigation has led to elevated levels of heavy metals in the soil and edible food in the areas in the vicinity of the industries.

Conclusion. The LIBS and TXRF results revealed high concentrations of Cr in soil and edible parts of the plants wheat and mustard, grown in agricultural fields near Jajmau industrial area, Kanpur, India. For the determination of Cr concentration, calibration curve using the spectral line at 267.7 nm by LIBS was found to be more suitable as compared to other Cr spectral lines. The calibration curve using atomic lines of Cr (like 425.4, 357.8 nm) can be used for the determination of concentration in the samples having lower amounts of chromium. The concentration of chromium was much higher than the defined nontoxic standard concentration of Cr in soil. Chromium concentrations in the seeds and leaves of wheat and mustard seeds were alarmingly higher than those prescribed by WHO and therefore were not suitable for consumption.

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