Remote Investigation of Total Chromium Determination in Environmental Samples of the Kombolcha Industrial Zone, Ethiopia, Using Microfluidic Paper-based Analytical Devices

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Abstract

Microfluidic paper-based analytical devices (μ-PADs) fabricated in Japan were employed for the determination of total chromium (Cr) in water, soil, and lettuce irrigated with wastewater in Ethiopia. The μ-PADs, which were printed by wax printing in Japan, were transported to Ethiopia followed by preparation for the determination of total Cr by adding appropriate reagents to the pretreatment and detection zones. Soil and lettuce samples were determined by the μ-PADs and a UV-Vis spectrophotometer in Ethiopia. The paired t-test showed that the mean total Cr concentrations determined in the soil and lettuce samples were not significantly different between μ-PADs and UV-Vis spectrophotometric analysis at the 5% level of significance. This implies that the μ-PADs has good accuracy and reliability and could be employed to monitor Cr in environmental samples. We found that the total Cr concentrations in all soil and lettuce samples were above the permissible limit. Moreover, the evaluation of Cr contamination level using the geo-accumulation index indicated that the soils are contaminated with Cr moderately to heavily. Thus, the present work successfully demonstrated the potential remote investigation of pollution in a less-equipped laboratory by transporting the μ-PADs fabricated in another laboratory.
Introduction

Heavy metals are a great threat to the environment and public health worldwide because they are non-biodegradable and persistent, and can accumulate in ecological systems.\textsuperscript{1-6} Chromium (Cr) has both beneficial and potential human risks,\textsuperscript{7, 8} as Cr(III) is an essential nutrient for maintaining lipid, insulin, and glucose metabolism\textsuperscript{7, 9} and has a low degree of human toxicity due to its low solubility and low absorptivity by organic molecules.\textsuperscript{10} Cr(VI) is the most toxic among the Cr species, as it is carcinogenic,\textsuperscript{9, 11, 12} irritant, and allergenic to humans.\textsuperscript{13} Risks to human health may occur due to excess exposure to trace elements either via the food chain or through direct exposure by dermal contact or ingestion of soil-borne trace elements.\textsuperscript{14} Intracellular chromate reduction allows Cr compounds to exert genotoxic effects.\textsuperscript{15} Cr(VI) in chromate form is isostructural with physiological sulfate and phosphate ions, and because of this molecular property, Cr(VI) readily enters cells of biological systems through the sulfate channels;\textsuperscript{16} subsequently, Cr(VI) undergoes a series of reduction reactions, yielding thermodynamically stable Cr(III). This intracellular chromate reduction is the activation event that is responsible for the generation of genotoxic damage.\textsuperscript{17} Therefore, monitoring the total Cr in environmental samples is an important safety issue.

Although spectroscopic and chromatographic techniques have been developed for the analysis of Cr, they are expensive and complex. Furthermore, the instruments are not easily portable and require professional personnel for operation. Therefore, there is a growing demand for new environmental monitoring approaches that are simple, fast, affordable, consistent with Green Chemistry principles,\textsuperscript{18} and available for use at the point of need. An emerging technology that may address this demand is microfluidic paper-based analytical devices (\(\mu\)-PADs), a new technology platform for extremely low-cost sensing applications.\textsuperscript{19-22} Recently, \(\mu\)-PADs have received considerable
attention since the World Health Organization (WHO) suggested that \( \mu \)-PADs are a promising platform to realize ASSURED policy. The policy states that diagnostic and health hazard monitoring devices for developing countries should be ASSURED: an acronym that means affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users.\(^{23}\)

The fundamental principle underlying microfluidic paper-based analytical devices (\( \mu \)-PADs) involves patterning a sheet of paper into hydrophilic channels bounded by hydrophobic barriers to create micron-scale capillary channels on paper. The pattern is generated by depositing a hydrophobic material, such as polymer or wax, on the paper, which serves as a barrier to guide the liquid wicking through microchannels.\(^{23-25}\) One of the widely-used techniques for creating hydrophobic barriers is wax patterning, which is rapid, inexpensive, and easy to be fabricated with minimal technical expertise.\(^{26}\) After fabrication of the \( \mu \)-PAD, colorimetric methods are frequently used to quantify the target analyte. The colorimetric methods are well established and are widely regarded as the most suitable detection technique to integrate with \( \mu \)-PADs due to their simplicity and compatibility with relatively low-cost reporting systems, including smartphones, digital cameras, and scanners.\(^{27-30}\)

Many colorimetric \( \mu \)-PADs have been developed to monitor metal ions.\(^{6}\) Mentele et al. developed colorimetric-based \( \mu \)-PADs for the analysis of Fe, Cu, and Ni in metal-containing aerosol samples.\(^{31}\) Chromogenic reagents for colorimetric detection of Fe, Cu, and Ni were loaded in the detection zone of the \( \mu \)-PADs while pre-conditioning agents were deposited in the channels between the sample and detection zones. Metal concentrations were quantified from the color intensity of the detection zone using a scanner in conjunction with image processing software.\(^{31}\) Rattanarat et al. introduced a multilayer \( \mu \)-PAD system that integrates colorimetric and electrochemical detection, i.e.,
colorimetric detection was used for quantifying Fe, Ni, Cr, and Cu in air samples while Pb and Cd were measured electrochemically in a separate layer. A distance-based colorimetric μPAD was also reported for the determination of Cu$^{2+}$. The developed method was applied for the analysis of real water samples and reported to be in good agreement with ICP-MS results. Meelapsom et al. developed silver nanoparticle-modified double-layer μ-PADs by the inkjet printing method for the detection of Hg$^{2+}$ ions from water samples. Ogawa and Kaneta used μ-PADs fabricated with a wax printer for the colorimetric determination of Fe$^{3+}$ in hot spring water. The determination of Cr was also reported using different types of μ-PADs by several researchers.

Most of the works related to μ-PADs are being carried out in developed countries that do not lack the resources necessary to conduct environmental monitoring with benchtop analytical instruments. However, the μ-PADs are more attractive in use for African countries because even conventional spectrophotometers are not available at many laboratories. To our knowledge, studies have not been conducted in Africa except for a group in South Africa working on point-of-care diagnostics to innovate and apply the μ-PADs for environmental monitoring of various pollutants in Africa. A dominant difficulty in the practical use of the μ-PADs in developing countries includes a fabrication method that frequently needs equipment inaccessible from these countries. Conversely, as described in the early study of the μ-PADs, one of the excellent characteristics is their transportability. Therefore, this study attempted to demonstrate the potential of the μ-PADs for the remote investigation of environmental pollution in Ethiopia by transporting the μ-PADs fabricated in Japan. The μ-PADs were transported from Japan to Ethiopia without depositing reagents of colorimetric detection for Cr. The colorimetric-based μ-PADs were prepared in Ethiopia and used for the first time to
determine total Cr in water, soil and plant samples collected from an industrial area of Kombolcha town in Ethiopia. Ultraviolet-Visible (UV-Vis) spectrophotometry was used to validate the μ-PAD-based method. The results from this study are very promising in terms of realizing the ASSURED policy set by the WHO, particularly in resource-limited settings, such as Ethiopia.

**Experimental**

**Apparatus**

Fundamentally, all experiments except for wax printing of the μ-PADs were conducted in Addis Ababa University. An electronic balance with ± 0.0001 g precision was used to weigh samples and chemical reagents. A drying oven (GX-65B), high-performance microwave digestion system (WAGTECH), UV-Vis spectrophotometer (HACHLANGE DR6000, Germany), and cell phone camera (TECHNO WX4 Pro, 8.3 Megapixels) were used for drying, digestion, absorbance measurement, and image capture, respectively. All the sample containers and tools, such as plastic bags, glassware, micropipettes, microwave digestion vessels, mortar, pestle, funnel, and spatulas were washed with detergent and tap water and then rinsed with 10% HNO₃ and double-distilled water.

**Device design and fabrication**

The μ-PADs were fabricated in Japan as follows: the pattern of the μ-PADs was drawn using Microsoft Office PowerPoint 2013 and printed on a sheet of filter paper (Chromatography Paper 1CHR, Whatman™, GE Healthcare Lifesciences, UK) using a wax printer (Xerox ColorQube 8580), followed by heating at 150 °C for 2 min in a drying machine (ONW-300S, AS ONE Corp.). The printed μ-PADs were transported to
Ethiopia where reagents for the determination of total Cr were added before the use.

**Reagents and solution preparation**

All reagents were analytical grade (Uni-Chem, NJ, USA) and used as received. A standard stock solution of 500 mg L\(^{-1}\) Cr was made by dissolving 0.30 g of ammonium dichromate in double-distilled water. A 10% nitric acid solution was prepared by adding 10 mL of concentrated nitric acid to double-distilled water and diluted to 100 mL with the same solvent. 1,5-Diphenylcarbazide (1,5-DPC) solution was prepared by dissolving 80 mg of 1,5-DPC and 170 mg of phthalic anhydride in 5 mL of methanol for both μ-PADs and UV-Vis spectrophotometric analysis. A Ce(IV) solution was prepared by dissolving 20 mg of cerium(IV) ammonium nitrate with enough double-distilled water to achieve a final volume of 100 mL. The buffer solution (pH 4.5) was prepared using 1 M sodium acetate and 1.67 M glacial acetic acid.

**Description of the study area**

Kombolcha is a fast-growing town in Ethiopia. It is approximately 380 km from Addis Ababa in the north-central part of Ethiopia. Kombolcha is one of the five national industrial development zones because of its close proximity both to the capital city of Ethiopia and the port in Djibouti. Consequently, the industrialization activity in Kombolcha town has been developing rapidly. However, for most industries, there is no proper waste treatment system. Effluent treatment predominantly takes place in lagoons or retaining ponds, but these facilities are quite old and designed to treat organic and sediment wastes only, rather than metal pollutants.44 The early established industries and the new industrial zones are located close to each other, but no joint efforts have been made by the industries to properly treat the generated waste. As shown in Fig. 1,
most industries, including steel processing, textile, tannery, and meat processing, discharge their effluents into the Leyole River whereas the brewery industry dumps effluent into the Worka River. These two rivers join the Borkena River, which passes through Kombolcha town and merges with the Awash River in the Afar region. These inputs may markedly increase the chemical pollution load in the receiving rivers.

Sample collection and pretreatment

The samples included soil, lettuce (*Lactuca sativa* L.), and water were collected from plots adjacent to the Borkena River at Kombolcha town in Ethiopia during the rainy season. A random sampling technique was used to collect representative samples from purposely selected sampling sites. Composite soil samples were collected from the supporting soil of lettuce plants at depths of 15 to 20 cm. The samples were placed in pre-cleaned polyethylene bags and bottles and then transported to the laboratory for further pretreatments. The plant samples were washed with tap water and rinsed with distilled water to remove extraneous substances, such as soil and dust particles, and finally chopped. Water samples were filtered through a 0.45 μm membrane filter, acidified with 10% HNO₃ during collection, and kept at 4 °C until analysis. The lettuce samples were first air-dried, then dried in an oven at 65 °C for approximately 2 h and finally powdered using a mortar and pestle. The soil samples were similarly air-dried and further dried in an oven at 105 °C for approximately 2 h. The dried samples were ground, homogenized, sieved using a 2 mm sieve, and kept in pre-cleaned plastic bags until digestion.

Digestion of soil and lettuce samples

Microwave digestion (EPA method 3052) was used for the digestion of soil and
lettuce samples. Samples of 0.5 g were weighed into each digestion vessel and digested using 7 mL concentrated HNO₃ and 1.5 mL of 30% H₂O₂. For method validation, 20 mg L⁻¹ standard solution was spiked into 0.5 g of the SS₁ soil sample. Each sample was digested in triplicate. The microwave power program was set to 100%, and the temperature was held at 200 °C for 10 min. After cooling, the samples were filtered through acid-washed (Whatman No. 1) papers and transferred into acid-washed 50 mL volumetric flasks. The digestion vessels were rinsed three times with double-distilled water, filtered, and transferred into 50 mL volumetric flasks, and the volume was adjusted to the mark using double-distilled water. Triplicate blank solutions were prepared following the same digestion procedure as the samples. The solutions were kept at 4 °C until analysis with the µ-PADs and UV-Vis spectrophotometer.

*Experimental procedure for the µ-PAD assay of total Cr*

The procedure reported by Rattanarat *et al.* was followed for the total chromium assay using µ-PADs. Thus, oxidizing and chromogenic reagents were loaded on the pretreatment and detection zones, respectively. Prior to reagent deposition, the backside of the µ-PAD was covered with clear scotch tape to prevent the solution from leaking out underneath the device. Using a micropipette, a 0.2 μL solution of ceric(IV) ammonium nitrate was added twice onto the pretreatment zone, and subsequently, a 0.2 μL solution of polydiallyldimethylammonium chloride (PDDA) (0.0025 M) was added to the same zone. The addition of PDDA prevents the complex from flowing to the edges of the hydrophilic channels because expansion to edges reduces measurement accuracy and sensitivity. Additionally, a 0.2 μL solution of 1,5-DPC and phthalic anhydride was deposited on the detection zone. During the standard and sample
analysis for the Cr assay, a 20 µL solution was loaded on the sample reservoir. Cerium(IV) was used to oxidize all forms of soluble Cr in the sample solution to Cr(VI) for reaction with 1,5-DPC.\textsuperscript{36} Subsequently, Cr(VI) reacted with 1,5-DPC to form 1,5-diphenylcarbazone (DPCO) and Cr(III). Finally, the resulting Cr(III) formed a distinct and intense purple color complex with DPCO.\textsuperscript{7, 36, 46}

This reaction is highly selective to Cr(VI) on the µ-PAD as demonstrated in the previous work which has shown no interferences of other metal ions including Fe(III), Ni(II), Cu(II), Al(III), Zn(II), Na(I), Co(II), Mg(II), Ca(II), Cd(II), and Mn(II).\textsuperscript{47} In terms of effect from humic substances, the microwave digestion decomposed them as seen in the color change of the soil samples. The soil samples dissolved in HNO\textsubscript{3} containing H\textsubscript{2}O\textsubscript{2} initially showed a yellow color which indicated the presence of humic substances. However, after microwave digestion, it turned into colorless which revealed the decomposition of humic substances. Therefore, the digestion process removed the influence of humic substances in this study.

As shown in Fig. 2, the pattern of the µ-PAD is tree-shaped, with narrow channels connected to a sample reservoir and detection zones. For preparing the pretreatment zone, a Ce(IV) solution was added and dried at the channel indicated by an arrow in Fig. 2. A sample solution was added at the same place as the pretreatment zone. The device includes five separate detection zones, four of which are used for quadruple measurement and one as a control. When the standard sample was loaded and distributed over five channels by capillary action, a purple color appeared in the detection zones (Fig. 2). Throughout the experimental procedures, the µ-PADs were allowed to dry completely between each reagent addition and before color intensity measurement.
Quantitative image processing

The developed color intensity was correlated with the target analyte concentration for quantification. A color image on the µ-PAD was captured by a cell phone camera without a flash to reduce bias from varying ambient light. A procedure reported by Mentele\textsuperscript{31} was followed for color intensity analysis by ImageJ software. The greyscale was set at 8 bit and adjusted based on the grey intensity to yield higher intensity values. Finally, a color threshold window was applied to effectively remove the wax background. For background measurements, color intensities for blank samples were measured using the same protocol described above. The background values were used to determine the baseline intensity for detection limit calculations. ORIGIN 8 and R software (R-3.3.2-win version) were used for the statistical analysis.

Results and Discussion

Analytical parameters for µ-PADs and UV-Vis spectrophotometry

The reaction between 1,5-DPC and Cr takes place with minimum interference from other substances.\textsuperscript{36, 45} However, the optimized amount of 1,5-DPC solution should be loaded to achieve appropriate sensitivity for the Cr assay. The addition of 1,5-DPC solution twice to the detection zone resulted in a more intense color image than a single addition, as shown in Fig. 3. However, triple deposition did not show a further increase in color intensity. Therefore, two deposits of 1,5-DPC solution represented an optimum amount used throughout the µ-PAD analysis.

In the µ-PAD analysis, a series of standard solutions of Cr(VI) (5, 10, 20, 35, and 100 mg L\textsuperscript{-1}) was prepared from a stock of 500 mg L\textsuperscript{-1} (NH\textsubscript{4})\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7} solution and analyzed to produce a calibration curve. The mean color intensities and the corresponding visual images for each standard solution are shown in Table 1. The color
intensity has an exponential dependence on concentration. A log-log graph results in a linear relationship in the form of a straight line. Therefore, the calibration curve was plotted as the log of mean color intensity versus the log of Cr mass added to the μ-PADs. This produced a linear response, in the range of 0.005-2 μg for the loaded 20 μL solution, with a correlation coefficient of 0.99499 and a linear equation of y=0.2032x + 2.2461.

In the UV-Vis spectrophotometer, the wavelength associated with maximum absorption (\(\lambda_{\text{max}}\)) was established at 540 nm by scanning a 6 mg L\(^{-1}\) standard solution of Cr(VI) treated with 1,5-DPC over a wavelength range of 340 to 900 nm. Thus, the absorbance was measured at 540 nm after 15 to 30 min of the reaction time to allow complete the reaction between 1,5-DPC and Cr(VI) in the acidic solution. Following the \(\lambda_{\text{max}}\) determination, a series of working standard solutions of Cr(VI) (0.2, 0.8, 2, 4, and 6 mg L\(^{-1}\)) was prepared from a stock of 500 mg L\(^{-1}\) (NH\(_4\))\(_2\)Cr\(_2\)O\(_7\) solution and analyzed to produce a calibration curve. The concentrations of the standard solutions were linearly related to absorbance values (R\(^2\) = 0.99681) and yielded a linear equation of y = 0.0396x + 0.0036 which was employed for the determination of the Cr content in real samples.

In both cases, the limit of detection was determined as the concentration which gives the mean signal intensity (color intensity or absorbance) plus three times the standard deviation for blanks. The results indicate that the limit of detection in the μ-PADs (0.005 μg or 0.25 mg L\(^{-1}\)) was higher than the limit of detection in UV-Vis spectrophotometry (0.06 mg L\(^{-1}\)). Although the μ-PADs are less sensitive than UV-Vis spectrophotometry in this study, the detection limit (0.25 mg L\(^{-1}\)) is below established regulatory limits, such as 2 mg L\(^{-1}\) for industrial discharge\(^{48}\) and 100 mg kg\(^{-1}\) for soil.\(^{49}\) This implies that the μ-PADs have adequate sensitivity to evaluate compliance with the
maximum allowable limits in industrial effluents and agricultural soil set by regulatory bodies. The analytical parameters obtained using the μ-PADs and UV-Vis spectrophotometry are summarized (Table 2) and compared with reported values. In Table 2, the limit of detection in the present μ-PAD assay is lower than those in previously reported studies\textsuperscript{32,36,50} except for that including a preconcentration method.\textsuperscript{38} Thus, the present study demonstrates that the μ-PAD analysis has good analytical parameters (better sensitivity and wider linear range) for the determination of total Cr in environmental samples.

\textit{Method validation and real sample analysis}

For every sample matrix analyzed, verification is required to ensure that neither a reducing condition nor chemical interference affects color development. As stated by the US EPA, the spike recovery must be from 85-115\% to verify the absence of interference and exhaustiveness of the sample preparation method.\textsuperscript{51} The quantity of Cr in the soil samples was quantified both by the μ-PADs and UV-Vis spectrophotometry. As shown in Table 3, the μ-PAD and UV-Vis spectrophotometry measurements yielded the percentage recoveries of 110 and 89\%, respectively. Both recoveries fall within the acceptable range. Furtherly, the reproducibility of the μ-PAD method was also evaluated for 6 replicate measurements of a standard solution at a concentration of 10 mg L\textsuperscript{-1}. Thus, the relative standard deviations for intra-day and inter-day was 3.7 and 4.1\%, respectively. Both relative standard deviations are less than 5\% which is an acceptable level of variability at the concentration level of mg L\textsuperscript{-1}. Therefore, these results collectively reveal that the μ-PADs have good accuracy and reliability and have the potential to be applied for real sample analysis.

The levels of a pollutant in soil, plants, and water are good indicators for
evaluating the effects of untreated industrial effluents on the nearby environment. Therefore, in this study, the levels of total Cr were determined in soil, plant, and water samples using the µ-PADs and UV-Vis spectrophotometry. The results from the water sample analysis were below the detection limits of both µ-PADs and UV-Vis spectrophotometry analysis, which could be due to the dilution effect, as the water samples were collected during the rainy season.

The concentrations of total Cr in the SS$_1$ and SS$_2$ soil samples analyzed by the µ-PADs and UV-Vis spectrophotometry are presented in Table 4. Although the µ-PAD results are greater than the UV-Vis results for both soil samples, a paired t-test indicates that there is no statistically significant difference between the two techniques at the 95% confidence level. This implies the potential suitability of the µ-PAD method for the analysis of Cr in environmental samples in the absence of benchtop analytical instruments, such as a UV-Vis spectrophotometer. This result suggests that the paper-based approach could be a good alternative or complementary device for detecting and monitoring environmental pollutants in developing countries.

The SS$_1$ soil sample was collected from a plot irrigated with a mixture of Leyole and Borkena river water, whereas the SS$_2$ soil sample was collected from a plot irrigated with Leyole River water. The Leyole River directly receives industrial effluents from tannery, meat processing, textile, and steel processing industries. Additionally, the Borkena River receives wastewater from households, hospitals, small- and large-scale enterprises, garages, and extensive car washes from the towns of both Dessie and Kombolcha. Soil samples were collected from the two sites, hypothesizing that there might be a difference in the level of chromium contamination between the two soil samples. However, the F-test followed by the t-test at the probability 0.05 level revealed that there is no statistically significant difference in the mean concentration of
total chromium between the two soil samples. From this result, it can be concluded that both sites were equally affected in terms of total Cr contamination, irrespective of the differences in the effluents input into the two rivers. The similarity in the effluents input into the two rivers and then into the irrigated soil could be the possible reason for the observed elevated concentrations of total Cr in both the SS₁ and SS₂ soil samples. A previous investigation by Zinabu et al. in the same study area also reported Cr concentrations as high as 64.600 mg L⁻¹ in the tannery effluent, which exceeded the US EPA emission guidelines.⁴⁴

Both the µ-PADs and UV-Vis spectrophotometry were also used for total Cr detection in leafy vegetable lettuce samples, and the results are also summarized in Table 4. Similar to the wastewater-irrigated soil analysis, the paired t-test showed that there is no statistically significant difference between the mean concentrations of total Cr obtained by the two methods, which once again reveals the reliability of the µ-PAD technique for environmental monitoring in resource-limited settings.

Likewise, there is no statistically significant difference (F-test, followed by t-test at 5% probability) in the mean concentrations of total Cr in the lettuce samples grown in the soils irrigated with river water. This is because the MP and LP lettuce samples were collected from soils (SS₁ and SS₂) with statistically similar loads of total Cr. Therefore, the same species of plant growing on a field with comparable total Cr concentrations would be expected to exhibit a similar uptake of Cr.

Assessment of total chromium pollution level and implications

As depicted in Table 4, all concentrations of total Cr in the soil samples were above the permissible limit (100 µg g⁻¹) set by the European Union Standards.⁵⁰ Cr contamination was also evaluated with respect to the global geochemical background
values in average shale. As reported by Ahmed and Amare and Guan et al., Muller introduced the geo-accumulation index ($I_{geo}$) to assess metal pollution in sediments. The geo-accumulation index has been applied in pollution studies for qualitative assessment of heavy metal contamination in soil. The $I_{geo}$ value is computed as follows: $I_{geo} = \log_2 \left( \frac{C_n}{1.5B_n} \right)$ where $C_n$ is the heavy metal concentration in the soil samples and $B_n$ is the geochemical background value of the heavy metal element in average shale. The constant 1.5 compensates for the natural fluctuations of a given metal and minor anthropogenic impacts. The geo-accumulation index of Cr in the irrigated soil samples of the present study is calculated with this equation, and the results are presented in Table 5. The results show that all the $I_{geo}$ values were higher than 2.4, which indicates that the irrigated soils are contaminated moderately to heavily by Cr derived from anthropogenic sources. The study revealed that the industrial activities around Kombolcha town are the main contributors to Cr contamination in the soil.

It is widely accepted that the risk of spreading trace element contaminants into the wider environment increases with their soil concentrations. Therefore, the high concentration of heavy metals in soils could be reflected by higher concentrations of metals in plants. In line with this, as shown in Table 4, the concentrations of Cr in the lettuce samples were approximately 10 times above the levels of the samples taken from another industrial area in Ethiopia (1.49 mg kg$^{-1}$ for tomato and 4.63 mg kg$^{-1}$ for cabbage). Leafy vegetables, such as lettuce and cabbage, uptake metals at higher concentrations than other vegetables because they require a higher transpiration rate to sustain their growth and moisture content. From the results of this study, it could be concluded that the practice of routine irrigation with water from rivers receiving industrial effluents and municipal wastewater has resulted in increased Cr
concentrations in the irrigated soil. Consequently, this contamination is manifested in lettuce and is becoming a threat to consumers. Therefore, to avoid excess accumulation of heavy metals in the body, the people living in the present study area should not eat large quantities of lettuce. In addition, industrial effluents and municipal wastes should be well treated before discharge into nearby rivers, and regular monitoring of Cr in effluents, sewage, rivers, soils, and vegetables should be carried out to prevent excessive accumulation of Cr in the food chain. Immediate intervention is also required to achieve safe levels of Cr in the soils and plants in the study area; particularly, the federal, regional, and local environmental authorities should monitor industrial pollution and evaluate compliance with the established standards.

Conclusions

The colorimetric µ-PAD method was used to quantify the levels of total Cr in soil, plant, and water samples, and the results were compared with those of UV-Vis spectrophotometric analysis. The trends of total Cr concentrations in the soil and lettuce samples were consistent between the results of both the µ-PAD and UV-Vis spectrophotometry that statistically exhibited no significant difference. This implies that the µ-PADs have the potential for environmental sample analysis and could substitute or complement conventional methods, especially in resource-limited countries. The µ-PADs are a promising alternative that could be portable to the point of measurement, particularly for water samples that require little sample preparation. The concentrations of total Cr in all soil samples were above the permissible limit set by the European Union Standards. The Cr contamination evaluated using the geo-accumulation index indicated that the wastewater-irrigated soils are contaminated with Cr moderately to heavily. Similarly, the concentrations of Cr in all lettuce samples
were approximately 10 times above the maximum permissible levels set by the FAO/WHO Joint Codex Alimentarius Commission, indicating a high degree of contamination in lettuce. Therefore, immediate intervention is required to attain safe levels of Cr in soils and plants; until then, the community should consume a limited amount of lettuce grown in the investigated area.

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Table 1 Mean color intensities of the standards with their corresponding visual images

| Mass of Cr added to µ-PADs (µg) with corresponding image | Mean color Intensity | log(mean color intensity) | log(µg Cr(VI)) |
|--------------------------------------------------------|----------------------|---------------------------|----------------|
| 0                                                      | 108.8±6.3            | 2.037                     | -1             |
| 0.1                                                    | 129.6±5.2            | 2.113                     | -0.7           |
| 0.2                                                    | 145.1±5.9            | 2.162                     | -0.4           |
| 0.4                                                    | 167.4±9.6            | 2.224                     | -0.15          |
| 0.7                                                    | 200.4±6.4            | 2.302                     | 0.3            |
| 2                                                      |                      |                           |                |
Table 2 Analytical parameters observed using µ-PADs and UV-Vis spectrophotometric analysis and comparison to other studies

| Method                      | Limit of detection | Linear range         | R²  | References  |
|-----------------------------|--------------------|----------------------|-----|-------------|
| µ-PAD (total Cr)            | 0.25 mg L⁻¹        | 0.25-100 mg L⁻¹      | 0.995 | Present work |
| UV-Vis spectrophotometry    | 0.06 mg L⁻¹        | 0.06-6 mg L⁻¹        | 0.997 | Present work |
| µ-PAD (total Cr)            | 0.12 μg (3 mg L⁻¹)ᵃ| 0.23–3.75 μg (5.75-93.75 mg L⁻¹) | 0.998 | Rattanarat et al.³² |
| µ-PAD (total Cr)            | 0.12 μg (2.4 mg L⁻¹)ᵇ | 0.38–6.0 μg (7.6-120 mg L⁻¹) | 0.998 | Rattanarat et al.³⁶ |
| µ-PAD (total Cr)            | 0.12 μg (3 mg L⁻¹)ᵃ| 0.15-6 μg Cr (3.75-150 mg L⁻¹) | 0.997 | Cate et al.⁵⁰ |
| µ-PAD (Cr(VI))              | 3 μg/L (0.003 mg L⁻¹) | 10–90 μg L⁻¹ (0.01-0.09 mg L⁻¹) | 0.993 | Alahmad et al.³⁸ |

ᵃ) 40 μL sample volume.
ᵇ) 50 μL sample volume.

R is the correlation coefficient.
Table 3: Determination of total Cr in spiked soil samples with μ-PADs and UV-Vis spectrophotometry

| Method   | Amount spiked (µg g⁻¹) | Amount found Mean ±SD | Amount determined before addition Mean ±SD | Amount recovered ±SD | Recovery % |
|----------|------------------------|------------------------|-------------------------------------------|-----------------------|------------|
| μ-PADs   | 360                    | 132.0±1.3              | 84.4±                                     | 397                   | 110        |
| UV-Vis   | 360                    | 1055                   | 735 ±53                                   | 320                   | 89         |

Mean image intensity

Concentration (µg g⁻¹)

Concentration intensity
Table 4 Concentrations of total Cr in the soil samples analyzed using µ-PADs and UV-Vis spectrophotometry

| Sample | µ-PADs (n=3) | UV-Vis (n=3) |
|--------|--------------|--------------|
|        | Intensity    | Concentration (µg/g) | Absorbance | Concentration (µg/g) |
| SS1    | 87.7±2.9     | 810±126       | 0.0343±0.0026 | 777±67 |
| SS2    | 119.2±0.5    | 737±14        | 0.0323±0.001  | 726±25 |
| MP     | 61.0±3.3     | 27.5±7.6      | 0.0116±0.0006 | 20.4±1.5 |
| LP     | 62.4±4.0     | 31.0±9.0      | 0.0120±0.0021 | 21.2±5.3 |

SS1 is soil irrigated with a mixture of Liyole and Borkena river water.

SS2 is soil irrigated with Liyole River water.

MP is Lettuce sample grown in soil irrigated with a mixture of Leyole and Borkena river water.

LP is Lettuce sample grown in soil irrigated with Leyole River water.
Table 5 Geo-accumulation index of Cr in wastewater-irrigated soil samples

| Method             | Sample | Cr concentration in sample (μg/g) | Geochemical background value of Cr in soil$^a$ (μg/g) | $I_{geo}$ |
|--------------------|--------|----------------------------------|------------------------------------------------------|----------|
| μ-PADs             | SS1    | 810                              | 90                                                   | 2.59     |
|                    | SS2    | 777                              |                                                      | 2.52     |
| UV-Vis spectrophotometry | SS1    | 737                              |                                                      | 2.52     |
|                    | SS2    | 810                              |                                                      | 2.43     |

$^a$ Global geochemical background value in average shale.$^{52}$
Figure Captions

Fig. 1 Location map of the study area (source: South Wollo Urban Development, Housing and Construction Office).

Fig. 2 μ-PADs. (a) Device with deposited reagents, (b) visual signal developed on device after introduction of a standard sample.

Fig. 3 Colour intensity on μ-PADs. (a) With single reagent deposition, (b) with two reagent deposition.
Fig. 1 Muhammed et al.
Fig. 2 Muhammed et al.
Fig. 3 Muhammed et al.
Graphical Index