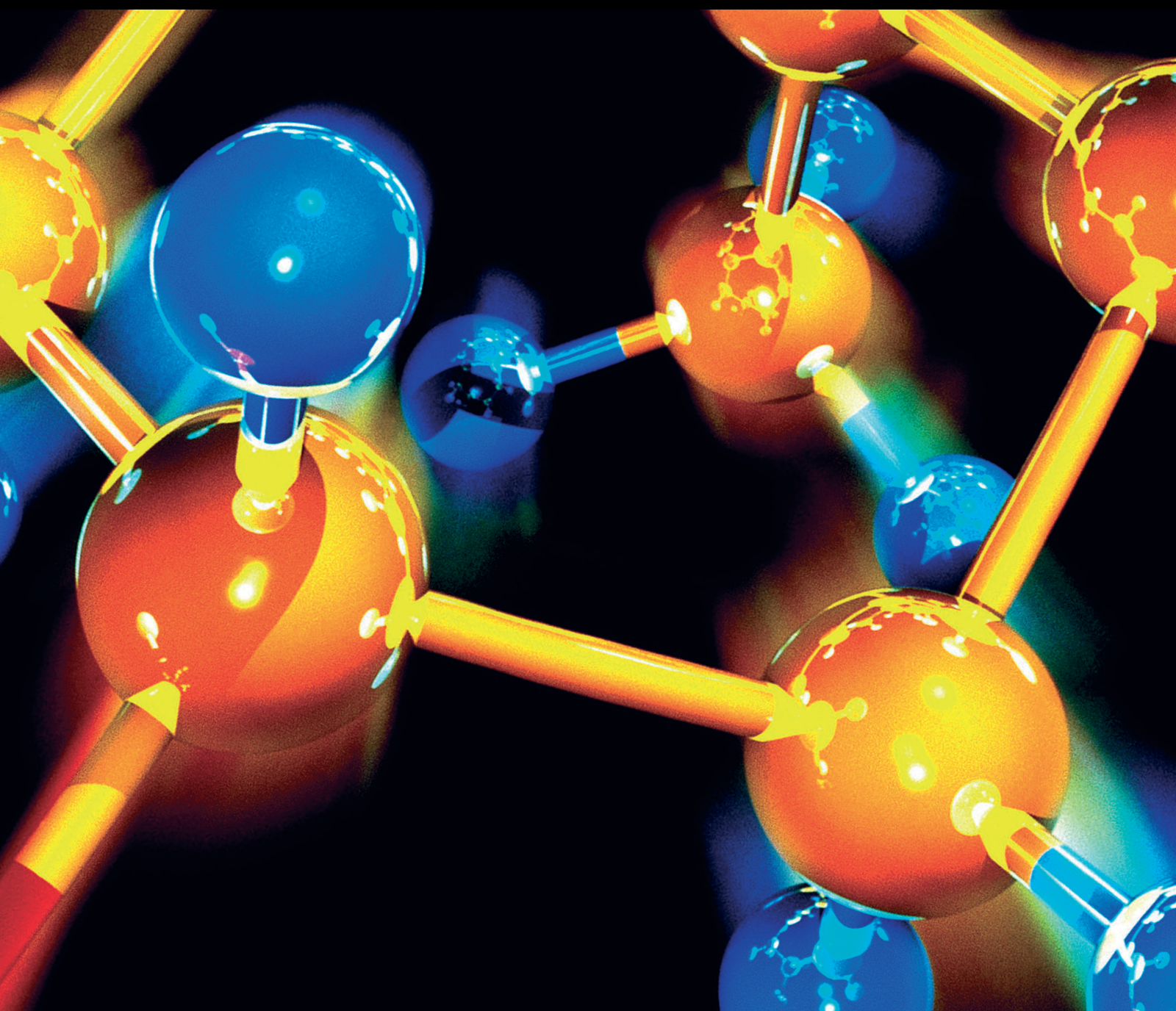# Toxic and Heavy Metals in Food Items

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Guest Editors: Abdul Hameed and Rima D. Alharthy
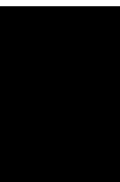
Toxic and Heavy Metals in Food Items

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Contents

Research Article

An Investigation for Heavy Metals' Contamination in Farmers' Fingernails: Case Study in Libya

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This study aims to assess the concentration levels of heavy metals, which are associated with health hazards: arsenic (As), cadmium (Cd), and lead (Pb) among Libyan farmers using fingernails as a biomarker. Factors that may contribute for accumulation of these toxic heavy metals in the farmers' fingernails were also evaluated. This cross-sectional study involved 127 farmers and 25 high school teachers living in the same geographical area as the farmers (served as the control group). Fingernail samples of the participants were collected, treated, and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for As, Cd, and Pb contents after microwave acid digestion. Results of this study indicated that the concentration levels of the investigated heavy metals in fingernail samples of both participating farmers and control group were in order of Pb > Cd > As. Also, the results showed that As, Cd, and Pb levels in the farmers' fingernails were found to be higher than in the control group by about 9-, 5-, and 2-fold, respectively. This difference for all heavy metals analyzed was found to be statistically significant ($P < 0.05$). As a conclusion, the results of this study clearly indicated that Libyan farmers have been exposed to high levels of toxic heavy metals as a result of their agricultural activities compared with the general population living in the same geographical area as farmers, which, in turn, pose a high risk to their health. Thus, toxicological, epidemiological, and clinical studies for the Libyan farmers are strongly recommended.

1. Introduction

Heavy metals have been associated with several diseases in epidemiologic research. They constitute significant potential threats to human health in both occupational and environmental settings [1–5]. Because heavy metals are non-biodegradable, this makes it predictable in the environment and they cause serious ecotoxicological problems [6]. Human exposure to heavy metal at low levels could cause poisoning and diseases, while accidental exposure at a high level could cause serious effect immediately [7]. Among the most studied and poisonous heavy metals are arsenic (As), cadmium (Cd), and lead (Pb) [8–11]. These heavy metals are extremely toxic even at low concentration level [8, 12]. Their toxicological profiles are ranked based on potential threat to human health; As is ranked 1st, Pb 2nd, and Cd 7th on the list of dangerous substance [13]. Several studies demonstrated that people who exposed to elevated level of As, Cd, and Pb often have serious health problems [10, 14–20]. According to the International Agency for Research on Cancer (IARC),

As, Cd, and Pb are classified as human carcinogens [9]. Thus, these heavy metals should not be present in the human body [21–23].

Estimation of heavy metal contents of exposed workers is essential to monitoring and detecting the impact of these heavy metals on human health [24, 25]. Human biomarker or biological marker is the most common application of biological monitoring for screening, diagnosis and assessment of metal exposures, and their risks as the results is normally accurate and reproducible [26–28]. Variety of biomarkers including hair, nails, blood, and urine specimens were previously used to assess heavy metals [29–43]. In the literature on biomarker of heavy metals, nails have many useful advantages for trace element analysis than other biological media [26, 29, 34, 38, 44–53]. Nails' tissues are rich in fibrous proteins that contain keratins as cysteine residues, and the changes in their appearance and composition are visible signs of deficiency or presence of stored substances in the body. Nails' roots are highly affected by health status of the cells, whereas blood and other body fluids give transient concentrations, and human nails represent longer term exposures of elemental concentration [54]. Furthermore, blood and other body fluids are not suitable to analyze levels of Cd because the metal exists briefly in the medium. Moreover, they are noninvasive yet convenient to collect, transport, and store, and grow more slowly compared to hair, not susceptible to external contaminants, unlike body fluids that are prone to contaminations [34, 55–59]. Therefore, nail tissue is more attractive diagnostic tool for monitoring human environmental exposure to the heavy metals.

Farmers have high risk of health problem because of exposure to the heavy metals during their agriculture activities such as pesticide and fertilizer processes. Several studies in different countries of the world demonstrated that working as a farmer is at risk of exposure to toxic heavy metals [60–62]. Following such observation, the assessment of toxic heavy metal levels for exposed farmers is essential in order to monitor and reveal these metals impact on their health. The Eastern region of Libya is considered an important agricultural area and number of farm owners are estimated at approximately 170000, and 40% of them consider agriculture as their full-time job. Unfortunately, in the past decade, diseases frequently showed excesses among Libyan farmers including leukemia and cancers of the skin (nonmelanocytic), lung, esophagus, stomach, colon, kidney, prostate, bladder, and brain and heart diseases as per the Libyan Ministry of health. Despite the seriousness of the problem, the causative factors that may lead to these excesses of diseases among Libyan farmers have not been known. Therefore, the aim of this study was to assess the concentration levels of heavy metals, which are associated with health hazards: arsenic (As), cadmium (Cd), and lead (Pb) among the farmers living in the Eastern region of Libya using fingernails as a biomarker. The study also extends to investigate factors that may contribute to the accumulation of As, Cd, and Pb in farmers' fingernails. This study is one of the first to examine toxic heavy metals exposure among Libyan farmers that will provide critical data on farming practices, exposure pathways, and how occupational exposure to heavy metals may affect farmers' health. Hence, the finding of this study will allow the agriculture sector to identify strategies to reduce heavy metal exposure, if any, in farmers and will potentially lay the groundwork for explanation the reasons that led to increase in the prevalence of diseases among farmers in the study area. In addition, the study can serve as an additional source of reference for future studies regarding occupational heavy metals exposure.

2. Materials and Methods

2.1. Chemicals and Standard Solutions. All the reagents used for sample preparation and digestion were of analytical grade (AR grade). Nitric acid (HNO$_3$, 65%), acetone, Triton X-100, and H$_2$O$_2$, 30%, have been bought from Merck (Darmstadt, Germany); the working standard solutions of As, Cd, and Pb have been prepared by a stepwise dilution of their certified standard stock solutions (Ultra grade, 1000 μgmL^{-1}, 5% HNO$_3$, ULTRA Scientific Analytical Solutions, USA). For accuracy, a human hair CRM (NCS DC 73347) from the China National Analysis Center for Iron and Steel (Beijing, China) has been used. Ultrapure water has been utilized for preparation and dilution of solutions. To avert contamination of the samples, all glass apparatus and digestion vessels have been washed with 1% v/v Triton X-100 and rinsed triple with ultrapure water followed by drenched in 10% v/v nitric acid for 12 hours. Then, all glass apparatus has been rinsed triple with ultrapure water and dehydrated.

2.2. Study Group. A total of 127 male farmers aged > 30 years participated in this study. The participating farmers had been working in agricultural fields as grain, vegetable, or fruit farmers for more than one year and some of them widely used pesticides and fertilizers in their agricultural activities. In order to obtain control group for the study, 25 male high school teachers aged also above 30 years and living in the same area with the farmers but not involved in any agricultural activities were selected.

2.3. Questionnaire. A validated questionnaire was given to each participant to provide the following information: the socio-demographic data, smoking habit, health status, and agricultural activities. An approval for the research has been obtained according to the Ethical Principles for Medical Research Involving Human Subjects. All participants' consents were obtained to take part in research after they were properly informed about its objectives and procedures. The participants for sampling were selected after the local approval.

2.4. Sample Collection. Samples of fingernails were collected from both farmer and control participants from the periods of May to September 2019. The ten fingernails of the participants were clipped at an average of two weeks after the interview and filling out the questionnaire. They were asked

to let their fingernails grow freely and not to use any special treatment. Before obtaining fingernails samples, participants washed their hands thoroughly and properly by using a medicated soap and ultrapure water, followed by tissue paper drying to remove any surface contaminant. On average, 0.5 to 1.0 g of fingernails samples has been collected from each participant utilizing sterilized stainless-steel clippers [63]. They have been kept separately in tightly sealed and labelled plastic bags, and each has been attached to participant's questionnaire until further analysis.

2.5. Sample Washing.

In this study, the fingernail samples were separately washed in accordance with washing method proposed by [50]. Briefly, they were washed and soaked in 1% v/v Triton X-100 for 2 hours, and then sonicated in an ultrasonic bath (Branson Ultrasonic Cleaner) for 20 minutes and for 15 minutes successively with acetone followed by ultrapure water, then again with acetone in ratio of 1 : 3 : 1, respectively. In every wash, 20 mL of solvent was used and decanted. After filtration with a filter paper, the fingernail samples were dehydrated overnight in an oven at 60°C and separately stored at room temperature.

2.6. Sample Digestion.

The fingernail samples were digested after drying following the procedure reported by [50] with little modification. They have been digested in a microwave system (Milestone Start D, Microwave Digestion System, VAC-1000, Italy). The choice of the microwave digestion process was based on its advantages of less acid usage, shorter time, and capability to keep volatile compounds in the solutions.

Accurately weighed 0.2 g of the dried fingernails samples has been placed in a dry, clean, Teflon digestion vessel, and 8 mL of an acid mixture of HNO_3 and H_2O_2 (3 : 1 ratio) has been added. Later, 4 mL of ultrapure water has been added. The digestion procedure has been continued for fifteen minutes at 25°C. The vessel has been then transferred to a microwave digestion system and operated at a power efficiency of 100% (600 W) in a three-step process and with a holding time of 10 min per step. The vessel has been cooled down and sonicated at the laboratory temperature for 30 min to clear nitrous oxide vapors. Ultrapure water has been used to wash the inside of the vessel. Using 1% HNO_3, each digested sample has been then quantitatively transferred into a volumetric flask and the volume has been made up to 100 mL with ultrapure water. The samples were then filtered through a 0.45-μm Millipore membrane filter, placed in plastic bottles, and stored at 4°C for a week and analyzed in triplicate by using ICP-MS. The reagent blanks, standard solutions, and standard reference material were similarly prepared and analyzed.

2.7. Sample Analysis.

The investigated heavy metals have been evaluated by using the ICP-MS (ELAN 9000, PerkinElmer SCIEX, USA). The instrument was first calibrated using the prepared standard solutions of As, Cd, and Pb before being optimized following the manufacturers'

instruction. Table 1 provides the operating conditions of the ICP-MS used for heavy metals determination in this study. Determination of each heavy metal concentration has been obtained by plotting a curve between intensities and concentrations after calibrating the instrument with a standard of known quantity. The ICP-MS instrument has been adjusted to measure the samples in triplicate. The heavy metals content of the samples has been reported as microgram per gram (μgg^{-1}).

2.8. Statistical Analysis.

In this study, the SPSS statistical software, version 22, has been used for all statistical analyses. The concentration levels of As, Pb, and Cd in investigated samples have been expressed as means ± SD. Significant differences between groups were subjected to one-way ANOVA followed by a Tukey–Kramer post hoc test. The level of significance was set at $P \leq 0.05$ with interval confidence of 95%. The Pearson method of correlation analysis was applied for examining heavy metals, age, and working period correlation.

3. Results

3.1. Analytical Method Characteristics.

Several analytical characteristics have been taken into account and evaluated for the validation of the ICP-MS analytical method for quantitative determination of As, Cd, and Pb in fingernail samples, namely, linearity, method detection and quantitative limits, recovery, and accuracy tests. A human hair CRM (NCS DC 73347) and working standard solutions of As, Cd, and Pb were used for validation measurement of ICP-MS. Five point's calibration curves were built up for each determined heavy metal. Linear range and correlation coefficient for calculating As, Cd, and Pb by ICP-MS have been shown in Table 2. Good linearity with correlation coefficients $(r) \geq 0.9995$ has been obtained for each determined heavy metal (Table 2).

For calculating the limits of detection (LOD) and limits of quantification (LOQ) for the studied metals analyzed with ICP-MS technique, a PerkinElmer method was applied [64]. The LOD of ICP-MS for blanks and the LOQ for the fingernails samples were low enough to detect the heavy metals at trace level in the fingernail samples (Table 2).

The recovery rates for the three heavy metals were carried out by spiking, in triplicate, the working standard solutions with different volume of each metal, to the fingernails samples. The spiked and unspiked samples were gone through same digestion and analysis by ICP-MS technique. The results of determinations exhibited good recoveries ranged from 92.3 to 98.3% as shown in Table 2. The good recovery values indicated no loss of the analyte occurred during preparation step of the sample and the sensitivity (intensity) was not affected by the fingernail samples matrix.

To investigate the validity of the applied technique, As, Cd, and Pb contents in human hair CRM (NCS DC 73347) have been determined. The experimental values were in accordance with certified values. The relative accuracy errors

TABLE 1: Operating conditions for ICP-MS analysis of As, Cd, and Pb in digested samples of fingernail.

Parameters	Condition
RF generator	40 MHz
RF power	1000 W
Spray chamber	Ryton scott
Nebulizer	Cross-flow
Plasma gas flow rate	15.0 l. min^{-1}
Auxiliary gas flow rate	1.0 l. min^{-1}
Nebulizer gas flow rate	0.60 l. min^{-1}
Sampler and skimmer cone	Nickel
Resolution	0.7 ± 0.1 amu
Dwell time	250 ms
Sweeps/Reading	20
Reading/Replicates	3

TABLE 2: Linear range, correlation coefficient, and limits of detection (LOD) and limits of quantification (LOQ) values for calculating As, Cd, and Pb using ICP-MS.

Heavy metals	Linear range (μgL^{-1})	Correlation coefficient (r)	LOD (μgg^{-1})	LOQ (μgg^{-1})	Recovery (%)	Relative accuracy error (%)
As	10 to 100	0.9995	0.0077	0.0258	98.3	1.22
Cd	10 to 100	0.9997	0.0019	0.0063	97.9	2.42
Pb	10 to 100	0.9998	0.0012	0.0038	92.3	3.28

of As, Cd, and Pb have been found to be 1.22, 2.42, and 3.28%, respectively (Table 2). The result indicated the validity (accuracy and precision) of the applied method (ICP-MS).

3.2. Participants' Characteristics.

The demographic characteristics of the control group and participating farmers were summarized in Table 3. This cross-sectional study involved 127 farmers and 25 high school teachers living in the same geographical area as the farmers (served as the control group). There is no statistical difference between control group and participating farmers with respect to the age.

Descriptions of the participating farmer are displayed in Table 4. As presented in Table 4, about 46.5% of the participating farmers were vegetable farmers, 23.6% fruit farmers, and 29.9% grains farmers, and had been working as farmers for more than one year. About 70.9% (90) of the farmers used pesticides and fertilizers, and 29.1% (37) did not use any type of pesticide and fertilizer; 22.0% (28) of the farmers used personal protective equipment (PPE) during their agricultural activities, while 78.0% (99) did not use any type of PPE. Of all the participating farmers, 56.7% were smokers and 35.4% have high pressure. A total of 25 male high school teachers also aged above 30 years served as the control group in this study.

3.3. Heavy Metals' Contents in Fingernail Samples.

Heavy metals contents in the fingernail samples of the participating farmers and control group are shown in Table 5 and Figure 1. The results revealed that levels of As, Cd, and Pb were found to be in order of Pb > Cd > As in fingernail samples of both the participating farmers and control group (Figure 1). It is noted that the levels of As, Cd, and Pb were found to be higher in fingernail samples of the participating farmers than those obtained from the control group by about 8.5-, 4.7-,

and 2.4-fold, respectively (Figure 1). This difference for all heavy metals analyzed was found to be statistically significant, $P < 0.05$ (Table 5).

3.4. Factors Contribute to Accumulate Heavy Metals.

In order to evaluate the factors could contribute to accumulate of the As, Cd, and Pb in the farmers' fingernails, data obtained from a relatively simple questionnaire survey (Table 5) were statistically analyzed. The findings of the effect of farmers' ages on the concentration levels of the studied metals in their fingernails are shown in Table 6. According to Table 6, it found that there were positive correlations between the farmers' fingernail heavy metals levels with respect to age ($P < 0.01$). Likewise, a positive correlation was showed between As, Cd, and Pb levels in the farmers' fingernails and their working period as farmers (Table 6).

The correlation between accumulation of As, Cd, and Pb concentrations in fingernails of the participating farmers and their usage of pesticides and fertilizers has been examined. The results are depicted in Table 7. The results show that the fingernail samples of the farmers who used pesticides and fertilizers during their agricultural activities have significantly higher As, Cd, and Pb contents than nonused farmers ($P < 0.01$).

In this study, the benefit of using personal protective equipment (PPE) to reduce the concentration levels of the As, Cd, and Pb in fingernails of the participating farmers has been estimated. The results are given in Table 8. The results clearly indicated that concentration levels of As, Cd, and Pb in fingernails of the participating farmers were tended to be decreasing with using PPE. The decreasing for all heavy metals analyzed was found to be statistically significant ($P < 0.01$).

Effect of the type of plantation on the accumulation of As, Cd, and Pb concentrations in fingernails of the

TABLE 3: Demographic characteristics of the control group and the participating farmers.

Demographic characteristics	Farmers	Control group
Number of participants (N)	127	25
Gender	Male	Male
Age (years)	$55.98 \pm 12.60^{*}$	$51.68 \pm 6.59^{*}$
Duration of working as a farmer (Years)	$26.89 \pm 13.57^{*}$	Nil

(*): Mean $\pm$ SD.

TABLE 4: Description of the participating farmers.

Social demographic data		Number of participants farmers (N)	Percentage %
Age (year)	>30	127	100
Duration of working as a farmer (year)	>1	127	100
Pesticides and fertilizers usage	Yes	90	70.9
	No	37	29.1
PPE usage	Yes	28	22.0
	No	99	78.0
Types of plantation	Vegetables	59	46.5
	Fruit	30	23.6
	Grain	38	29.9
Smoking habit	Yes	72	56.7
	No	55	43.3
Blood pressure status	Normal	82	64.6
	High	45	35.4

TABLE 5: Concentration levels of heavy metals in fingernails samples of the participating farmers and the control group.

Heavy metal	Subjects	Number of participants (N)	Level of heavy metals (μgg^{-1})		Significance tests at $P \leq 0.05$				
					F-test		t-test		
			Mean $\pm$ SD	Range	Calculated value	Critical value[1]	Calculated value	Critical value[2]	P-value
As	Farmers	127	3.016 ± 1.857	0.083–8.223	35.752	0.038	15.983	2.609	$P < 0.05$
	Control group	25	0.354 ± 0.116	ND–0.429					
Cd	Farmers	127	9.059 ± 4.026	0.122–18.003	43.607	0.038	17.613	2.609	$P < 0.05$
	Control group	25	1.912 ± 0.963	0.222–3.388					
Pb	Farmers	127	11.322 ± 3.785	0.319–21.111	46.548	0.038	8.230	2.609	$P < 0.05$
	Control group	25	4.747 ± 2.847	0.453–8.399					

ND: not detected (lower than the LOD of $0.0077 \mu gg^{-1}$ for As).
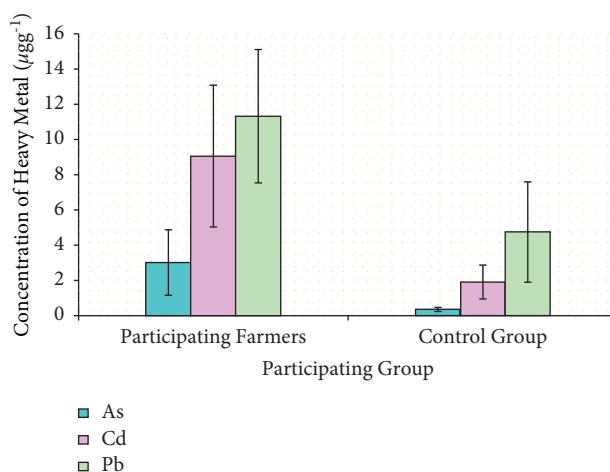

FIGURE 1: Concentration levels of heavy metals in fingernail samples of the participating farmers and the control group.

TABLE 6: Correlation between concentrations of As, Cd, and Pb in fingernails of Libyan farmers and their age and working period.

| Heavy metal | Correlations, r | |
	Age	Working period
As	0.376**	0.357**
Cd	0.382**	0.355**
Pb	0.254**	0.191*

Correlation is significant at the 0.01 level ($P < 0.05$). (**) Correlation is significant at the 0.01 level ($P < 0.01$).

TABLE 7: Concentration of As, Cd, and Pb in fingernails of the participating farmers according to pesticides and fertilizers usage.

| Heavy metal | Concentration levels of studied heavy metals (μgg^{-1})* | | |
	Use of pesticides and fertilizers ($N = 90$)	Not use of pesticides and fertilizers ($N = 37$)	P-value
As	3.504 ± 1.694	1.826 ± 1.714	($P < 0.01$)
Cd	10.052 ± 3.644	6.642 ± 3.924	($P < 0.01$)
Pb	12.475 ± 2.691	8.519 ± 4.567	($P < 0.01$)

(*): results are expressed as mean ± SD. N: number of participants.

TABLE 8: Concentration levels of As, Cd, and Pb in fingernail of participating farmers according to PPE usage.

| Heavy metal | Concentration levels of studied heavy metals (μgg^{-1})* | | |
	Use of PPE ($N = 28$)	Not use of PPE ($N = 99$)	P-value
As	1.678 ± 0.990	3.393 ± 1.886	$P < 0.01$
Cd	6.064 ± 1.891	9.905 ± 4.070	$P < 0.01$
Pb	9.749 ± 2.946	11.767 ± 3.889	$P < 0.01$

(*): results are expressed as mean ± SD. N: number of participants.

participating farmers have been also evaluated. The results have been presented in Table 9 and Figure 2. The results of the study indicated that the type of plantation highly contributed to elevate levels of As, Cd, and Pb in the fingernails of participating farmers (significantly different ($P < 0.01$)). The highest concentration of the studied heavy metals has been found in fingernail of vegetable farmers, followed by fruit and grain farmers (Figure 2).

The impact of smoking habit on the accumulation of As, Cd, and Pb in fingernails of the participating farmers has been investigated (Table 10). The results in Table 10 showed insignificant difference ($P > 0.05$) in concentration levels of As, Cd, and Pb among smokers and nonsmoker farmers.

In the present study, the correlation between fingernails As, Cd, and Pb concentration levels and the blood pressure of participating farmers are examined and summarized in Table 11. The results showed that the blood pressure of participating farmers insignificantly correlated ($P > 0.05$) with the concentration levels of studied heavy metals (Table 11).

4. Discussion

Farmers may exposure to several potentially hazardous chemical and biological substances during their agricultural activities including irrigation using wastewater, pesticides, fertilizers, insecticides, solvents, fuels, and oils [65–67]. These agricultural activities may release some toxic heavy metals such as arsenic (As), cadmium (Cd), and lead (Pb) to farming communities that lead to adverse effects to farmers'

health [32, 60–62, 68, 69]. These heavy metals are regarded as human carcinogen from extremely low levels of exposure, having no possible beneficial metabolic functions for humans.

Vomiting, diarrhea, and abdominal pain are the immediate symptoms of acute arsenic toxicity. These symptoms are often followed by numbness of the extremities, muscle cramps death, in extreme cases [70, 71], whereas increased risks of reproductive health problems, such as miscarriage, preterm delivery, stillbirth low weight at birth, and declining fertility in males are associated with chronic exposure to As [72–74]. Diabetes, cancer, and neurological disorders, such as learning and behavioral defects, are reported as well [3, 75]. Occupational exposure to Cd is associated with increased risk of lung [19] and prostate cancers [76, 77], as well as reduced pulmonary function [78]. Cumulatively, exposure to Cd is associated with kidney disease, bone damage, impairment of Zn metabolism, low level of hemoglobin and hematocrit, gastritis, vomiting, diarrhea, high mortality of cancer, and increased risk of cardiovascular disease [3, 14, 15, 79–82].

In regard to Pb toxicity, brain and kidney damage, muscular weakness, hypertension, and arthritis are the common findings [83, 84]. Mental retardation and birth defects are reported as well [84]. Therefore, it is critical to investigate the concentration levels of toxic heavy metals in the farmers' fluids and tissues. Nails as a human biomarker are one of the most accurate and precise application for biological monitoring and estimating of metal exposures [22, 29, 31, 32, 35, 39, 41, 49]. They represent long-term

TABLE 9: Concentrations of Cd, Pb, and As in fingernails of farmers in various plantation groups.

| Heavy metal | Concentration levels of studied heavy metals (μgg^{-1})* | | | |
	Vegetable farmer ($N = 59$)	Fruit farmer ($N = 30$)	Grain farmer ($N = 38$)	P-value
As	3.832 ± 1.703	2.971 ± 1.451	1.785 ± 0.712	$P < 0.01$
Cd	10.697 ± 3.664	9.046 ± 3.157	6.528 ± 3.934	$P < 0.01$
Pb	12.911 ± 2.558	12.004 ± 1.958	8.317 ± 4.674	$P < 0.01$

(*): results are expressed as mean $\pm$ SD. N: number of participants.
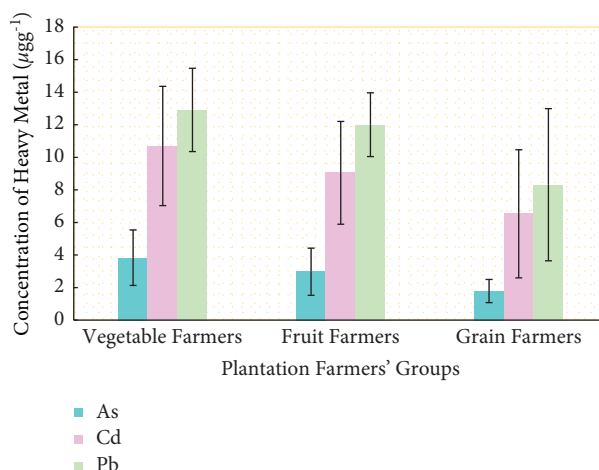
FIGURE 2: Concentration of studied metals in fingernails of farmers in various plantation farmers' groups.

TABLE 10: Concentrations of As, Cd, and Pb ($\mu g\ g^{-1}$) in farmers' fingernail samples according to smoking habit.

| Heavy metal | Concentration levels of studied heavy metals (μgg^{-1})* | | |
	Smokers ($N = 72$)	Nonsmokers ($N = 55$)	P-value
As	3.146 ± 1.863	2.844 ± 1.855	0.367
Cd	9.356 ± 3.945	8.669 ± 4.121	0.343
Pb	11.822 ± 3.344	10.668 ± 4.238	0.099

(*): results are expressed as mean $\pm$ SD. N: number of participants.

TABLE 11: Concentration levels of As, Cd, and Pb in farmers' fingernail samples according to their blood pressure.

| Heavy metal | Concentration levels of studied heavy metals (μgg^{-1}) | | |
	Normal blood pressure ($N = 82$)	High blood pressure ($N = 45$)	P-value
As	3.277 ± 1.887	2.872 ± 1.838	0.242
Cd	9.518 ± 4.081	8.807 ± 3.997	0.343
Pb	11.934 ± 3.598	10.987 ± 3.865	0.178

(*): results are expressed as mean $\pm$ SD. N: number of participants.

exposures of elemental concentration as reported by several studies over the literature [26, 29, 40, 41, 45, 48, 49, 58].

Nowadays, several diseases such as cancers were noted increasing among the Libyan farmers compared with the population living in the same region according to the Libyan Ministry of Health. This study was carried out to estimate the concentration levels of heavy metals, which are associated with health hazards: As, Cd, and Pb among farmers living in the Eastern region of Libya to provide some information about levels of these selective heavy metals. Factors that may contribute to accumulate these heavy metals in the farmers'

body were also investigated. A total of 127 farmers and 25 high school teachers living in the same geographical area as the farmers (served as the control group) were participated in this study. The fingernails as biomarker were collected from the participating and analyzed by the validated ICP-MS method for As, Cd, and Pb contents as previously mentioned.

Results obtained in this study revealed that the fingernail heavy metals levels were found to be in order of Pb > Cd > As in both the participating farmers and the control group (Table 5). This result clearly indicated that both participating

groups have been exposed to higher level of Pb than the two toxic heavy metals under study (Figure 1). However, the results are not surprising as Pb has been described as the most common and harmful environmental contaminant to arise in human civilization. The high accumulation of Pb could have resulted from the exposure to Pb-contaminated food and drinking water. Production of batteries, smelting and metal plating process, and exhaust from vehicles, pigment additives, gasoline, fertilizers, and herbicides are also the possible sources of Pb exposure in the environment [3, 13].

The concentration levels of As, Cd, and Pb in fingernail samples of both the participating farmers and the control group were statistically compared at 0.05 probabilities. It was found that the levels of As, Cd, and Pb among the participating farmers were found to be higher than those in the control group by about 8.5-, 4.7-, and 2.4-fold, respectively (Figure 1). This difference for all heavy metals analyzed was found be statistically significant, $P < 0.05$ (Table 5). With the absence of any mining factory around the agricultural area where both the farmer and the control group living, the high concentrations of studied toxic heavy metals in the fingernails of the participating farmers might be attributed to occupational exposures such as indiscriminate use and improper application of pesticides and fertilizers in the agriculture, which may lead to contaminate the farmers' communities.

Since there were no available data about levels of heavy metals among Libyan farmer populations, the results were compared with other similar studies from other countries (Table 12). As can be seen from Table 12, arsenic (As) level was found to be higher than the studies carried out in Qatar [62], and lower than that in Malaysia and Carolina, USA [60, 61]. However, cadmium (Cd) and lead (Pb) in the present study showed elevated level comparing with other studies in Qatar, Malaysia, and Carolina, USA. As human nails are chemically same, the difference in fingernail heavy metal levels in different countries might be related to the different environmental exposure and geographic conditions.

Heavy metals generally accumulate in the human body and reflect the body's biomedical and environmental history as well as long-term metabolic changes. Previous studies have mentioned that several factors are contributed for accumulation of heavy metals in farmers' body including the farmers' age, their working periods as farmers, use of pesticides and fertilizers, type of plantation, use of personal protective equipment (PPE), smoking habits, and their blood pressure levels [32, 60–62, 68]. Therefore, in this study, the factors that may contribute for accumulation of As, Cd, and Pb in farmers' fingernails were evaluated.

Statistical analysis in this study showed that the age of the participating farmers was affected positively in accumulation of heavy metals in farmer's fingernails (Table 6). The positive relationship pointed out that the concentration levels of As, Cd, and Pb in the farmers' fingernails increased with farmers' age ($r = 0.376$, 0.382, and 0.254 for As, Cd, and Pb, respectively). The significant correlation ($P < 0.01$) between the studied heavy metals and age revealed that, for the most

participating farmers that were examined, the older participants have been exposed to elevated amount of heavy metals for most of their adult life. This finding agrees well with other authors [61, 62], who found that an increase in the heavy metals levels as farmers' age increase. Likewise, a positive correlation ($r = 0.357$, 0.355, and 0.191 for As, Cd, and Pb, respectively) between working period as a farmer and concentration levels of studied heavy metals in fingernails of the farmers was also observed (Table 6). This result accords with that obtained by other investigators, who reported that the duration of work as farmer has significant influence to the level of heavy metals exposure [62].

Table 7 summarized the correlation between accumulation of As, Cd, and Pb concentrations in fingernails of the participating farmers and their usage of pesticides and fertilizers. The results showed that the fingernail samples of the farmers who used pesticides and fertilizers during their agricultural activities have significantly higher As, Cd, and Pb contents than nonused farmers. It is likely that the widespread use of pesticides and fertilizers significantly contributed to the level of studied metals in fingernails of the farmers. This effect was also noted by other investigators [60], who reported that the exposure of farmers to pesticides and fertilizers during their agricultural activities contributed positively to the concentration of studied metals in their bodies.

The purpose of using personal protective equipment (PPE) is to protect people specially farmer from exposing to heavy metals and prevent them from entering the body. The collected information from questionnaires showed that only 22.0% of the participating farmers used PPE during work (Table 4). Therefore, benefit of using PPE to reduce the concentration of the As, Cd, and Pb in fingernails of the participating farmers has been estimated. The results clearly indicated that concentration of As, Cd, and Pb in fingernails of the farmers statistically tends to decrease with using PPE (Table 8). This finding accords with other investigators, who reported that the using PPE seems to be very important factor to reduce and control the concentration of these metals in farmers' bodies [60].

The results of the present study revealed that vegetable farmers exhibited the highest concentrations of As, Cd, and Pb in their fingernail samples, while grain farmers recorded the lowest levels (Table 9). However, from the participating farmers' questionnaires, it was noted that all the vegetable farmers used pesticides and fertilizers, and the grain farmers, on the contrary, used less pesticides and fertilizers during their agricultural activities. This might be the reason for the increasing heavy metal exposure in vegetable farmers rather than fruit, and grain farmers.

The impact of smoking habit on the accumulation of As, Cd, and Pb in fingernails of the participating farmers has been investigated (Table 10). Several studies revealed that tobacco smoke contained considerable amount of As, Cd, and Pb, and thus, it increases their levels in smokers [61, 79, 85–89]. By contrast, the results of present study show insignificant difference ($P > 0.05$) in concentration of As, Cd, and Pb among smokers and nonsmoker farmers. However, this result agrees well with that obtained by other investigators who reported that the As, Cd,

TABLE 12: Meta-analysis of heavy metals (As, Cd, and Pb) levels in farmers' fingernail samples.

Heavy metal	Present study	Other studies		
	Libya*	Qatar** [62]	Malaysia* [60]	Carolina, US** [61]
As ($\mu g g^{-1}$)	3.016 ± 1.857	0.26 (0.04–5.73)	7.801 ± 3.184	13.23 (11.11–15.35)
Cd ($\mu g g^{-1}$)	9.059 ± 4.026	0.03 (<0.01–0.27)	0.874 ± 0.746	0.20 (0.81–0.21)
Pb ($\mu g g^{-1}$)	11.322 ± 3.785	0.51 (0.10–11)	6.611 ± 5.170	1.26 (1.08–1.43)

(*): results are expressed as mean ± SD. (**): results are expressed as mean and range.

and Pb concentrations were not influenced by smoking farmer [60].

Several epidemiological studies showed that exposure to As, Cd, and Pb can injure vascular system causing many types of diseases such as high blood pressure [58, 90, 91]. In the present study, the correlation between As, Cd, and Pb concentration levels and the blood pressure of the participating farmers have been examined (Table 11). The results show that the blood pressure of participating farmers insignificantly correlated ($P > 0.05$) with the concentration of studied metals. Same results have been obtained by [60], who did not find any significant difference in the studied metals concentration among people with normal blood pressure and high blood pressure.

5. Conclusion

In the present study, the As, Cd, and Pb levels in fingernail samples collected from Libyan farmers were examined in order to evaluate exposure levels to these toxic heavy metals and to explore the factors related to such exposure. The results of this study showed elevating levels of As, Cd, and Pb in the fingernails of the Libyan farmers compared with the general population living in the same geographical area and the fingernails accumulated metals in the order Pb > Cd > As. Statistical analysis indicated that the farmer's age, working period as farmer, and pesticides and fertilizers usage were highly contributed to increase the concentration levels of As, Cd, and Pb in fingernails of the participating farmers. On the other hand, PPE use was significantly decreasing the concentrations of As, Cd, and Pb in fingernails of the participating farmers. The results also exhibited a significant variation in the concentrations of heavy metals in association with types of plantation, where vegetable farmers showed the highest concentrations of As, Cd, and Pb in their fingernail compared with those in fruit and grain farmers. No effect of smoking habit or farmer's blood pressure was observed on the concentrations of As, Cd, and Pb in the fingernails of the participating farmers. As a conclusion, the results of this study proved that Libyan farmers have been exposed to high levels of some toxic heavy metals as a result of their agricultural activities compared with the general population living in the same geographical area, which, in turn, pose a high risk to their health. Thus, those farmers should be kept aware about the dangers relevant to these types of agricultural activities to avoid health issues resulted from the continuous exposure. Furthermore, toxicological, epidemiological, and clinical studies for the Libyan farmers are strongly recommended.

Data Availability

The data used to support the findings of the study are included within the article.

Conflicts of Interest

The authors declared that they have no conflicts of interest to any party.

Acknowledgments

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Research Article

Acridine-2,4-Dinitrophenyl Hydrazone Conjugated Silver Nanoparticles as an Efficient Sensor for Quantification of Mercury in Tap Water

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Excretion of heavy metals especially mercury ($Hg^{2+)}$) from the industries into the environment becomes a major global problem. In this context, mercury is a highly dangerous metal which poses serious impact on human health. In the present study, acridine-(ACR-) based silver nanoparticles (ACR-AgNPs) were prepared and employed as a nanosensor for effective detection and quantification of Hg^{2+} in tap water. Conjugation between ACR-based coating agent and silver was examined by UV-visible and FT-IR spectroscopy, while morphology and particle size were determined through atomic force microscopy (AFM), dynamic light scattering (DLS), and scanning electron microscopy (SEM). Furthermore, sensing behavior of nanosensor for metal ions was evaluated by mixing different metals such as Mn^{2+}, Ni^{2+}, Ba^{2+}, Mg^{2+}, Cr^{3+}, Pb^{2+}, Pd^{2+}, Al^{3+}, Sn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+}, and Hg^{2+} with ACR-AgNPs. Among all the added metal ions, only Hg^{2+} resulted in significant quenching in the absorption intensity of ACR-AgNPs. The limit of detection of the ACR-AgNP-based nanosensor was found to be $1.65\,\mu M$ in a wide pH range (1-14). The proposed mercury sensor worked efficiently in the presence of other interfering agents such as other metal ions. Therefore, the synthesized ACR-AgNPs have proved to be an efficient and robust nanosensor for quantitative detection of Hg^{2+} in real sample analysis such as tap water. The proposed method does not require expensive instrumentation and trained manpower.

1. Introduction

The excretion of heavy metal from the industries in the environment has become a major global problem. Mercury (Hg^{2+}) is one of highly toxic pollutant which is found in soil, water, and air [1]. It is a highly dangerous metal and causes serious health problems to humans and animals. Its direct contact with eyes results in vision loss.

The human central nervous system is badly affected by action of Hg^{2+} ions, if it presents in the blood. It also induces lung problem, the person feel breathing problem that leads to kidney failure and to ultimate death [2–8]. It has been estimated that the total amount of Hg added from industries into the environment is about 5,000–8,000 metric tons per year [9, 10]. Various techniques have been employed for detection of Hg^{2+} ions in the soil,
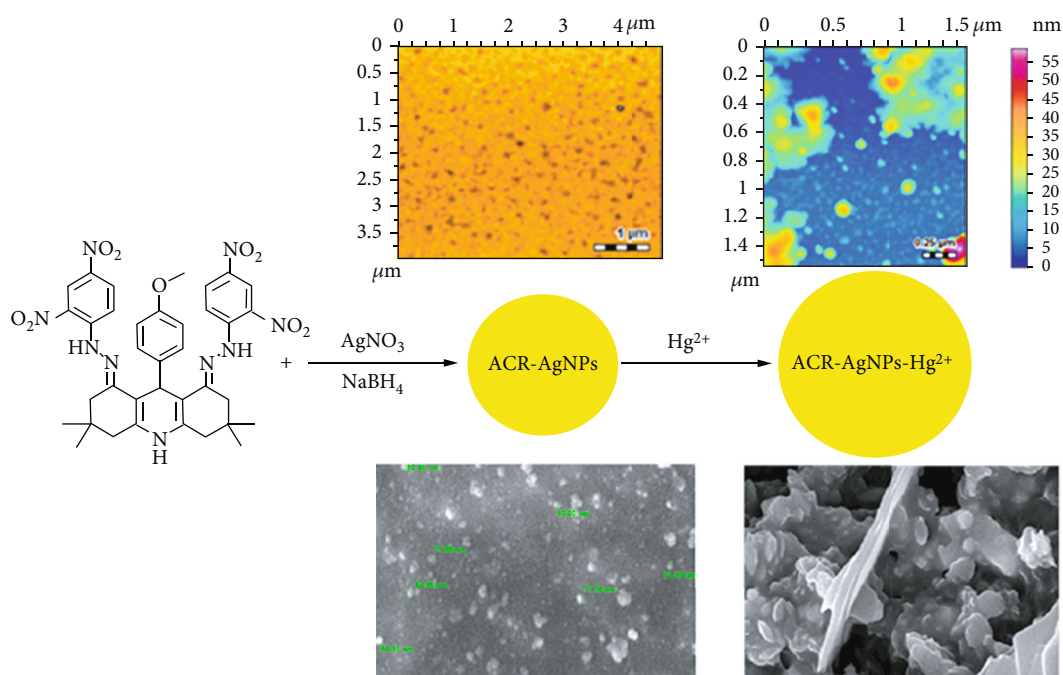
FIGURE 1: Graphical presentation of present study.

water, and even in the air that include atomic absorption or emission spectroscopy (AAS/AES) [11, 12], X-ray absorption spectroscopy (XAS) [13], inductively coupled plasma mass spectrometry (ICP-MS) [8], and atomic fluorescence spectrometry (AFS) [14]. Previous methods are difficult, costly, require expensive instruments, and also expertise of manpower to run these instruments. Therefore, it is necessary to develop an easy, economic, selective, and sensitive method for detection of mercury in the environmental samples. Recently, fluorescent and colorimetric sensors are found to be easily approachable, and facile method for the detection of environmentally pollutant metal ions (Hg^{2+} ions) [15–19]. Due to presence of electronic configuration of d10 orbital of mercury, there is no spectral signature that inhibits its practical applications [20–22]. However, due to having this unique feature (Hg^{2+} ions), the nanosensor has great optical response to detect Hg^{2+} ions present in biological samples or in the environment [22–24]. The nanosensors have remarkable ability to detect mercury ions (Hg^{2+}) even if it is present in the very small amounts. In this context, silver nanoparticles of chemosensors were synthesized which possess strong optical response in the spectral range of 400 to 480 nm. The addition of external substances in the AgNPs results in the spectral shift (bathochromic, hyperchromic, hypsochromic, and hypochromic) due to interaction between external species with silver nanoparticles [25–27]. Moreover, nanoparticles of silver are cheap, nontoxic, and environment friendly. Furthermore, these have great capability to detect the heavy metal ions (Hg^{2+} ions) and have high selectivity and sensitivity depending upon the nature of the used stabilizing agent. Various methods are present to synthesize AgNPs such as chemical reduction method, ion sputtering, and sol gel [28–30]. The abovementioned methods engage

harmful chemicals and also need high energy which may cause decomposition of the chemosensors [31, 32]. Due to the nontoxic nature and biological applications, the silver nanoparticles have great attention for the scientists, chemists, and pharmaceutics [33–35]. Moreover, silver nanoparticles provide remarkable opportunity to detect the heavy metal ions, dyes, pesticides, fertilizers, bacteria, fungi, and drugs in the environmental and biological systems.

Acridines derived from anthracenes belong to the class of heterocyclic compounds [36]. It is formed when two rings fuse in a central position to a pyridine ring, also recognized as dibenzo-pyridine. Derivatives of acridine are well-known for their remarkable pharmaceutical and biological properties [37–39]. Interesting molecular structure of acridine derivatives raised our interest to explore their potential as sensors. Therefore, we targeted to prepare silver nanoparticles by using (1E,8E)-1,8-bis(2-(2,4-dinitrophenyl) hydrazono)-9-(4-methoxyphenyl)-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8,9,10-decahydroacridine (ACR) as stabilizer and to explore their potential for sensing Hg^{2+} in environmental samples. Different spectroscopic techniques were carried out for the characterization of acridine-based silver nanoparticles such as UV-visible and FT-IR spectroscopy, while morphology and particle size were observed through atomic force microscopy (AFM), dynamic light scattering (DLS), and scanning electron microscopy (SEM). The detection and quantification of mercury ions are the major target of current study (graphical description, Figure 1).

2. Methods and Material

2.1. Materials and Instrumentations. All the chemicals used were of analytical grade and were procured from Sigma
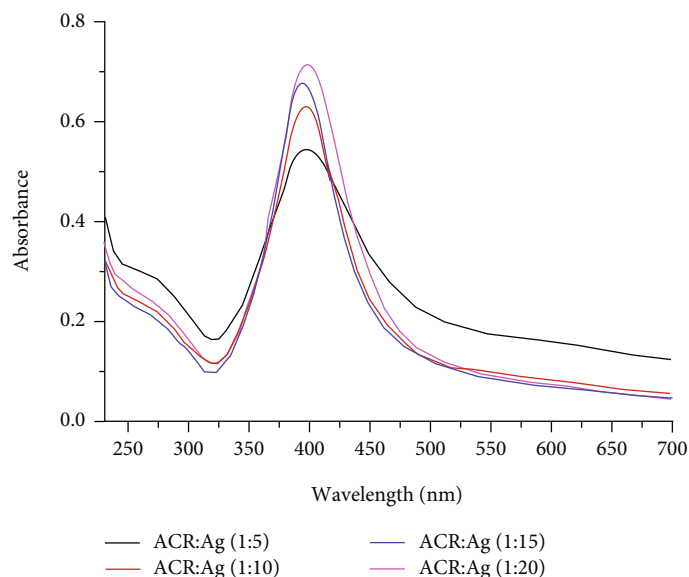
FIGURE 2: UV-visible spectra of acridine-coated silver nanoparticles (ACR-AgNPs).
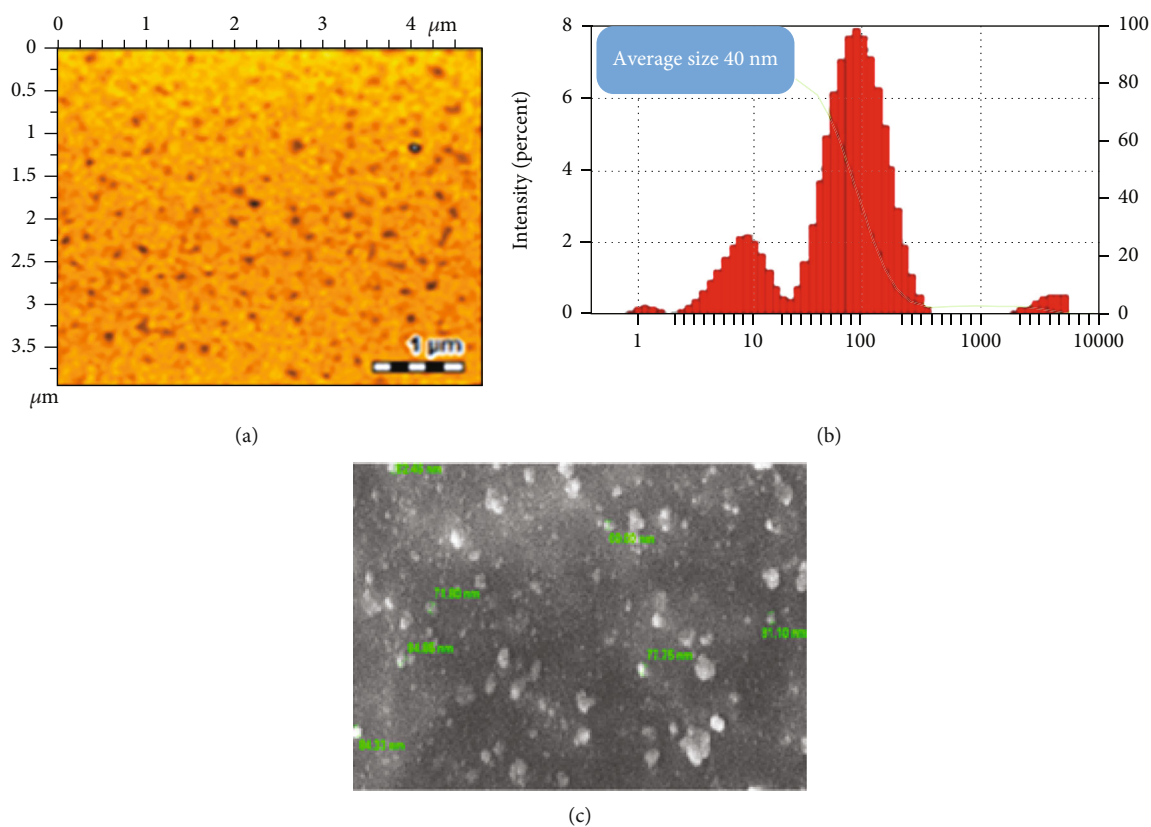

(a)

(b)

(c)

FIGURE 3: (a) Two-dimensional AFM images of ACR-AgNPs. (b) The percent intensity size distribution of ACR-AgNPs. (c) SEM image of ACR-AgNPs.

Aldrich. All the solvent used in this study were analytical grade and were purchased from Riedel-de Haen. The UV-visible spectroscopy was conducted on Shimadzu-240 Tokyo, Japan, having a one-centimeter quartz cuvette. The morphology of samples was determined with atomic force microscopy (Agilent-5500, USA). A diluted drop of sample was put on the mica, air dried, and analyzed in contact mode. The average size and zeta potential were observed by zeta sizer. The measurement of pH of the sample was done through model 510 pH meter (Oakton, Eutech), having Ag/AgCl (reference) electrode and a glass (working) electrode.
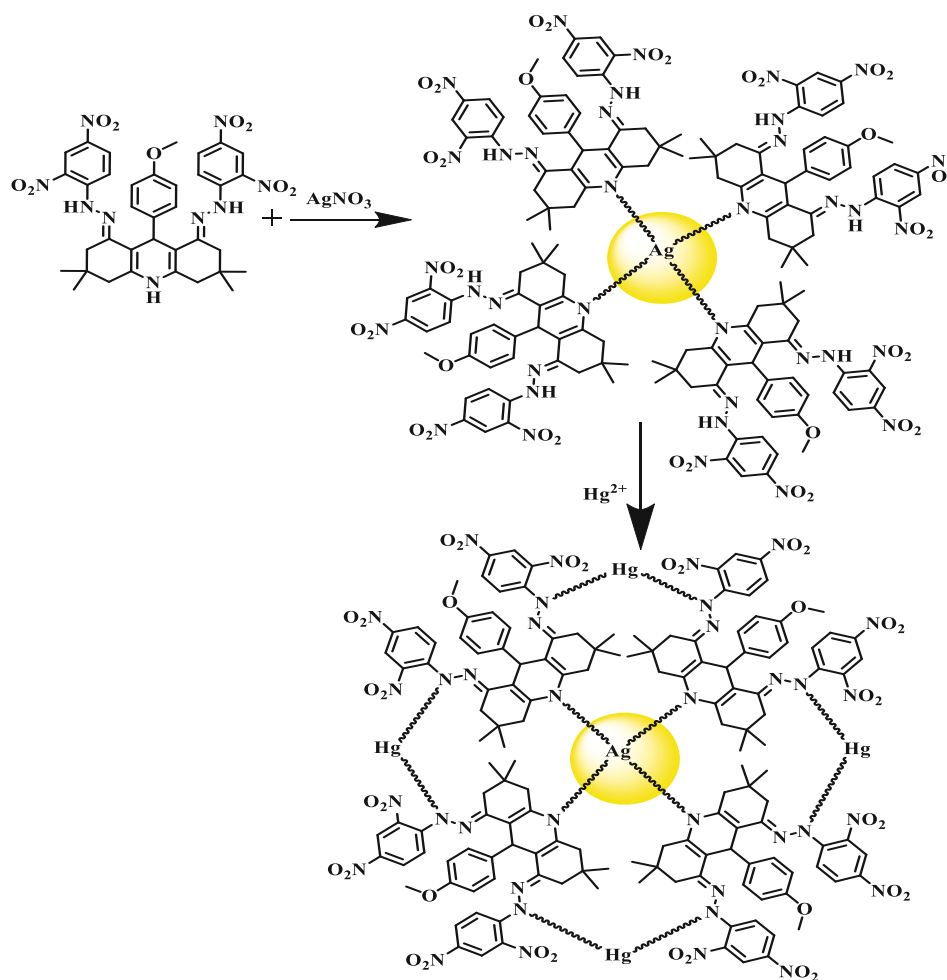
FIGURE 4: Schematic representation of nanoparticles synthesis and complexation with Hg^{2+}.

2.2. Synthesis and Characterization of ACR. (1*E*,8*E*)-1,8-bis(2-(2,4-dinitrophenyl)hydrazono)-9-(4-methoxyphenyl)-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8,9,10-decahydroacridine (ACR) was synthesized by mixing benzaldehyde, ammonium acetate, dimedone, and nickel (II) fluoride tetrahydrate (catalyst) in ethanol. After maintaining acidic medium by using acetic acid, hydrazine hydrate, phenyl isothiocyanate, and 2,4-dinitrophenyl hydrazine were added. Details of synthesis and characterization of ACR have been given in our recent publication [40].

2.3. Synthesis of ACR-AgNPs. ACR-AgNPs were synthesized by chemical reduction method using sodium borohydride as a reducing agent. Stock solution of silver nitrate (1 mM) was prepared in deionized water which was further diluted to 0.1 mM. A 1.0 mM solution of ACR was prepared in ethanol and further diluted up to 0.1 mM. To synthesize ACR conjugated silver nanoparticles (ACR-AgNPs), equimolar solutions of synthesized ACR (0.1 mM) and silver nitrate (0.1 mM) were mixed in several ratios (1 : 5, 1 : 10, 1 : 15, and 1 : 20) and stirred at room temperature for10 minutes. After that, few drops (5-6) of $NaBH_4$ (4 mM) were added and resulted solution was stirred further. After 30 minutes

of continuous stirring, yellow color appears in the initially colorless solution indicating the formation of colloidal silver nanoparticles. The synthesized ACR-AgNPs were centrifuged at 12000 rpm for 12 min until pallets were obtained, after discarding supernatant followed by freeze drying to obtain solid ACR-AgNPs. The obtained nanoparticles were stored at 4°C for further use.

2.4. Morphological Analysis by AFM. For determination of particles size and surface morphology of the synthesized ACR-AgNPs. For the analysis of the complexation between ACR-AgNPs and Hg^{2+}, both the solutions were mixed in 1 : 1 ratio (*v/v*). For AFM sample preparation, 10 μL freshly prepared solution of ACR-AgNPs was placed on a cleaned mica disc and dried in air. After complete drying, AFM analysis was performed in tapping mode.

2.5. FTIR Analysis. FT-IR spectra in the range 400-4000 cm^{-1} were recorded by FT-IR 8900 Shimadzu using KBr disc. For FTIR analysis of ACR-AgNPs, 5 mg dried nanoparticles were mixed with a small amount of KBr powder and grounded to a fine disc. Similarly, 5 mg of ACR-AgNPs powder mixed with equal ratio with Hg^{2+} was mixed with a small
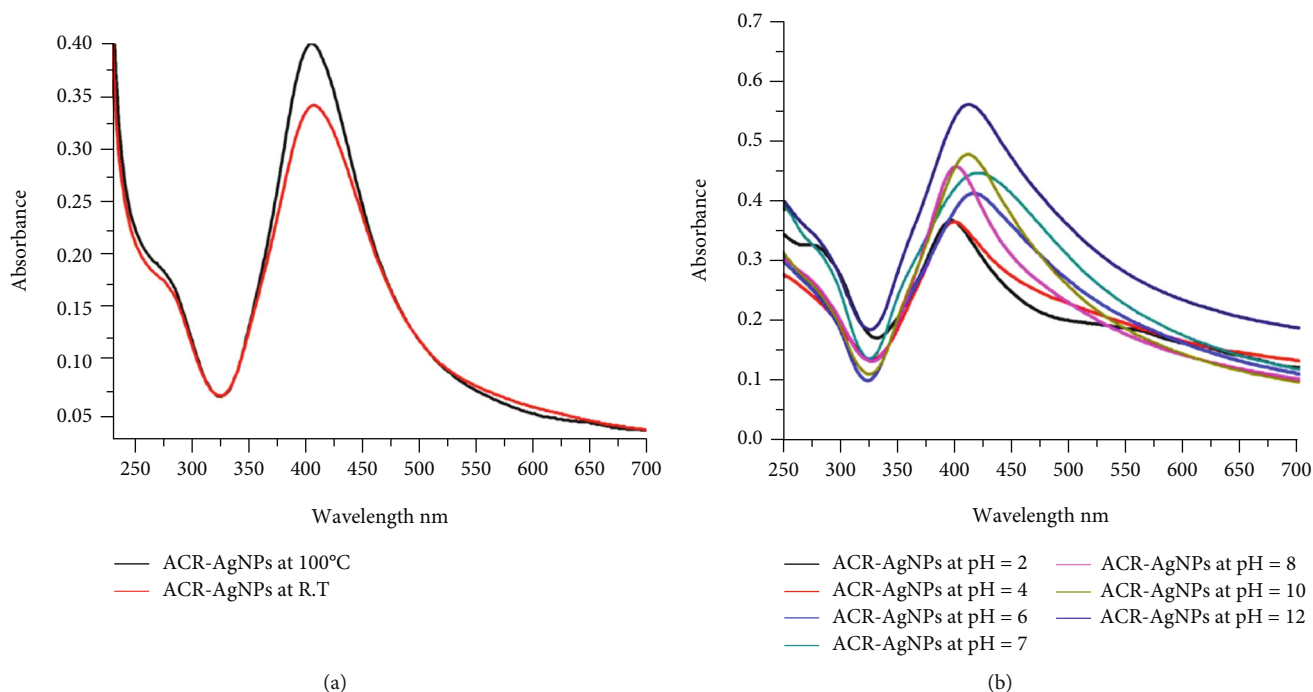
(a)

(b)

FIGURE 5: Stability of ACR-AgNPs. (a) Effect of temperature treatment. (b) Effect of pH on the stability of ACR-AgNPs.

amount of KBr and grounded to a fine disc. Thereafter, analysis was done by FT-IR 8900 Shimadzu, Japan.

2.6. General Methods for Sensing Experiments.

The photophysical potential of ACR-AgNPs towards metal ions was explored using UV-visible spectroscopy. For general screening experiments, 1.0 mL of freshly prepared ACR-AgNPs solution was mixed with various metal ions (100 μM), and changes in the absorption spectrum were recorded. For the determination of limit of detection for Hg^{2+}, concentration-dependent experiments were performed, various concentration of Hg^{2+} (5-100 μM) were treated with ACR-AgNPs. The limit of detection for Hg^{2+} was calculated using standard deviation of blank and slope of straight-line equation using following formula LOD = $3.3 \times$ S.D/Slope. For competitive analysis, several interfering metal ions were tested in presence of Hg^{2+}, and obtained results were compared with that of Hg^{2+} alone.

2.7. Spiking in Tap Water.

100 μM solution of mercury was prepared in laboratory tap water taken from the University of Karachi. The freshly prepared ACR-AgNPs were mixed with mercury solution of equal concentration and equal quantity prepared in tap water. UV-visible spectrum was recorded and compared with spectrum recorded in deionized water.

3. Results and Discussion

3.1. Synthesis of ACR-AgNPs.

UV-visible spectra were recorded to finalize the suitable ratio for optimum stabilization of ACR-AgNPs. Spectral analysis showed that ACR to $AgNO_3$ ratio of 1:10, 1:15, and 1:20 gave a sharp peak as compared to the ratio 1:5 at 410 nm (Figure 2). Peak broadness may lead to incorrect results as the addition of any interfering substance especially low concentration may not induce a significant response. Thus, ratio 1:20 was used for the synthesis of ACR-AgNPs because in this ratio the more nanoparticles are formed and the peak is sharp and of high intensity.

3.2. Characterization of ACR-AgNPs.

The morphology and size of ACR-AgNPs were examined by two-dimensional and three-dimensional images obtained by atomic force microscopy (AFM), which showed spherical particles in the size range of 40 to 45 nm (Figure 3(a)). And also the SEM (scanning electron microscopy) analysis of the same revealed the spherical shape of the ACR-AgNPs having a size in similar range (Figure 3(c)).

Size distribution of ACR-AgNPs is examined through dynamic light scattering (DLS) in terms of percent intensity, ACR-AgNPs showed the average size of 40 nm and polydispersity index (PDI) of 0.556, Figure 3(b). FTIR spectra of ACR and ACR-AgNPs were recorded between 4000 and 500 cm^{-1}. Spectra showed prominent infrared absorbance peaks at 1618 cm^{-1}. Stretching vibration of carbon-carbon single and carbon-carbon double bond (aromatic) causes absorbance bands at 1585, 1618, 1477, and 1423 cm^{-1}. Asymmetric stretch of ^-C–O^- and CH_3 (bend) is observed at 1129 and 1253 cm^{-1}. Whereas peaks at 1511 and 1330 cm^{-1} indicate C-N and N-H stretching of aromatic amine. Stretching vibrations of secondary amine (N$^-$H) group of acridine showed a broadband at 3300 cm^{-1} while a sharp band appeared at 3644 cm^{-1}. In spectrum of acridine conjugated silver nanoparticles, the -N-H band shifted from 3300 cm^{-1} to 3443 cm^{-1}. These findings suggest that the secondary amine nitrogen of ACR takes part in the stabilization of silver nanoparticles (Figure 4) [41, 42].

3.3. Stability of ACR-AgNPs. Stability of nanoparticle under different conditions plays critical role in application of nanoparticles to the real samples. In order to determine the stability of newly synthesized ACR-AgNPs, different parameter was optimized such as pH of the medium and effect of temperature on the stability of nanoparticles. In order to determine the effect of temperature on the stability of nanoparticle, ACR-AgNPs were boiled to 100°C, cooled to room temperature, and spectra were recorded. As can be seen from Figure 5(a), ACR-AgNPs are found to be more stable after temperature treatment. The effect of pH on the stability of ACR-AgNPs was also optimized as shown in Figure 5(b). The newly prepared AgNPs stabilized by ACR were found to be highly stable in a wide range of pH (2-12).

3.4. ACR-AgNPs as Sensor. As a real target of this study, the effect of addition of commonly found ions/metal ions such as NH_4^+, Mn^{2+}, Ni^{2+}, Ba^{2+}, Mg^{2+}, Cr^{3+}, Pb^{2+}, Pd^{2+}, Al^{3+}, Sn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+}, and Hg^{2+} in ACR-AgNPs is evaluated. UV-visible spectra of the ACR-AgNPs after addition of metal salt solutions (100 μM) in 1:1 ratio is recorded (Figure 6). Addition of mercury solution in ACR-AgNPs resulted in decrease in the absorbance intensity that could be linked to the binding of Hg^{2+} with the nitrogen of ACR-AgNPs by their lone pairs. As depicted in Figure 4 most probably, the Hg^{2+} forms stable complex with Schiff base nitrogen atom of ACR-AgNPs. After the complex formation with Hg^{2+}, the electronic environment of ACR-AgNPs is disrupted and consequently absorption intensity decreased significantly. All other tested metals did not have any pronounced effect on the SPR band of ACR-AgNPs. The interaction of Hg^{2+} with ACR-AgNPs can be monitored by several factors like surface modification, aggregate morphology, particle size, and shape [43].

Furthermore, sensitivity of ACR-AgNPs for Hg^{2+} was checked in the presence of other metal ions viz. NH_4^+, Mn^{2+}, Ni^{2+}, Ba^{2+}, Mg^{2+}, Cr^{3+}, Pb^{2+}, Pd^{2+}, Al^{3+}, Sn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+}, and Hg^{2+} by adding ACR-AgNPs and Hg^{2+} along with all the aforementioned metals. The change in the absorption intensity of ACR-AgNPs+Hg^{2+} upon addition of various other competing metal ions was recorded and compared with the intensity obtained of the same without interferent. The presence of other metal ions has no significant effect on the quenching of SPR band as obtained for the same amount of mercury without interferent, Figure 7. This indicates that the proposed sensor has efficiently detects the Hg^{2+} ions in the real sample even in the presence of other metal ions.

In order to determine stoichiometry of ACR-AgNPs and Hg^{2+}, Job's method was used in which total concentration was kept constant, while mole fraction of Hg^{2+} was increased gradually from 0.1–1. Values of absorption intensities at 410 nm revealed that minimum absorbance was obtained at mole fraction value of 0.6, indicating 1:1 binding ratio of ACR-AgNPs with Hg^{2+} (Figure 8).

In addition, sensing behavior of synthesized nanosensor at elevated concentration of Hg^{2+} was checked between
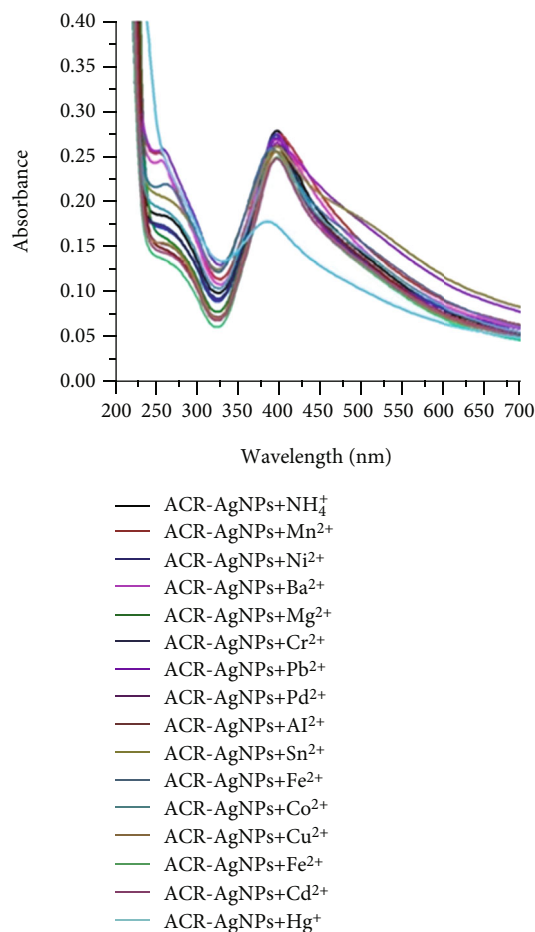

FIGURE 6: Change in the absorption intensity of ACR-AgNPs upon addition of different metal cations (100 μM).

ranges of 5-100 μM Hg^{2+} solutions (Figure 9). An efficient and concentration-dependent decrease in the absorbance intensity of ACR-AgNPs is observed. A linear relation was observed with the increasing concentration of mercury with R^2 value of 0.9893. The limit of detection for mercury ion was found to be 1.65 μM.

3.5. Characterization of ACR-AgNP-Hg^{2+} Complex. The morphology and size of ACR-AgNPs and Hg^{2+} complex were observed through AFM and DLS and SEM (Figure 10). Images obtained through AFM and SEM showed spherical and large size particles. Percent intensity of Hg^{2+} complex of ACR-AgNPs indicated increment in particle size from 40 to 90 nm with a polydispersity index (PDI) of 0.577. Moreover, analysis through AFM, SEM, and DLS revealed increment in size which confirmed ACR-AgNPs complex formation with Hg^{2+}.

The absorbance bands of ACR-AgNPs complex with Hg^{2+} obtained in the range of 1000–4000 cm^{-1} are 3443, 1614, 1205, and 1138 cm^{-1} (Figure 11). Among these, broad absorbance band at 3443 cm^{-1} is due to the bending vibration of secondary amine (-NH). Whereas Hg^{2+} binding to the nitrogen of aromatic amine and oxygen of hydroxyl results in shifting of this band from 3443 to
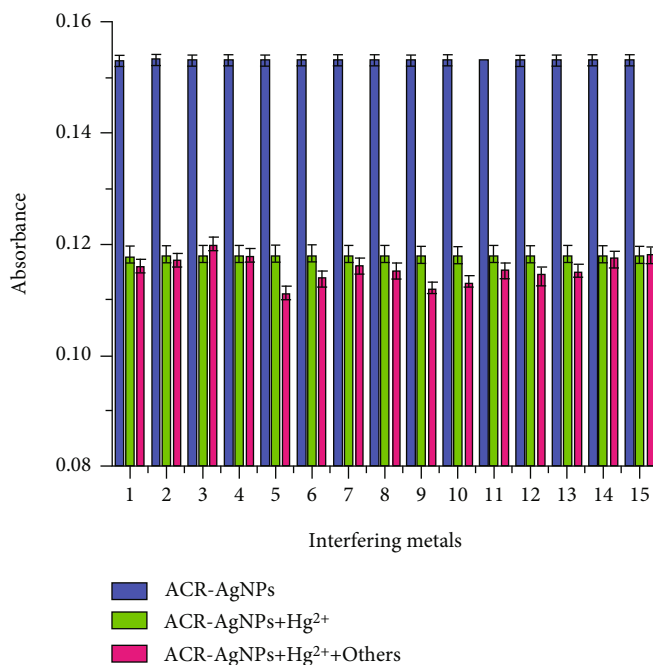
FIGURE 7: Consequence of competing metal ions on the sensing performance of ACR-AgNPs for Hg^{2+}, in the presence of 1: NH_4^+, 2: Mn^{2+}, 3: Ni^{2+}, 4: Ba^{2+}, 5: Mg^{2+}, 6: Cr^{3+}, 7: Pb^{2+}, 8: Pd^{2+}, 9: Al^{3+}, 10: Sn^{2+}, 11: Fe^{2+}, 12: Co^{2+}, 13: Cu^{2+}, 14: Fe^{3+}, and 15: Cd^{2+} at 410 nm.
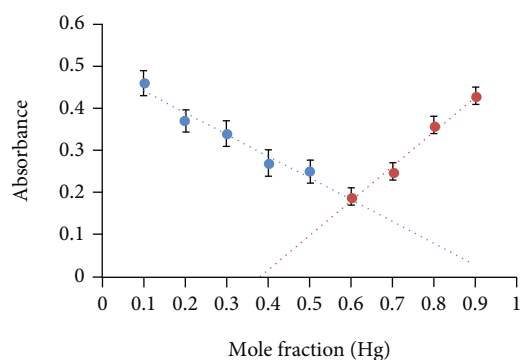
FIGURE 8: Job's plot to observe binding stoichiometry of ACR-AgNPs complex with Hg^{2+}.

FIGURE 9: Change in the absorption intensity of ACR-AgNPs upon addition of varied concentrations of Hg^{2+} (5-100 μM).

$3580 \, cm^{-1}$ and also decreased broadness of the absorbance band appeared at $3580 \, cm^{-1}$, because in the complex, both amine and hydroxyl groups of ACR-AgNPs are involved in chelation with Hg^{2+}. The peak at $1687 \, cm^{-1}$ is due to C=N in ACR-AgNPs and in case of its complex with Hg^{2+}, a sharp peak at $1614 \, cm^{-1}$ was observed. Moreover, C-N and C-O-C stretching bands were obtained at $1205 \, cm^{-1}$ and $1138 \, cm^{-1}$ (Figure 11).

It is generally required to evaluate effect of pH on the host guest interaction in a complex in context of real sample applications. To determine an optimized pH for spectroscopic analysis, stability of ACR-AgNPs complex with Hg^{2+} in the pH range of 2-13 is evaluated (Figure 12). To maintain the pH of solutions, the dilute sodium hydroxide and hydrochloric acid was added drop wise.

At pH range of 2-6, the decrease in intensity of the ACR-AgNPs complex with Hg^{2+} might be associated with deprotonation of ACR-AgNPs. Conversely, the absorption intensity of ACR-AgNPs complex with Hg^{2+} remained
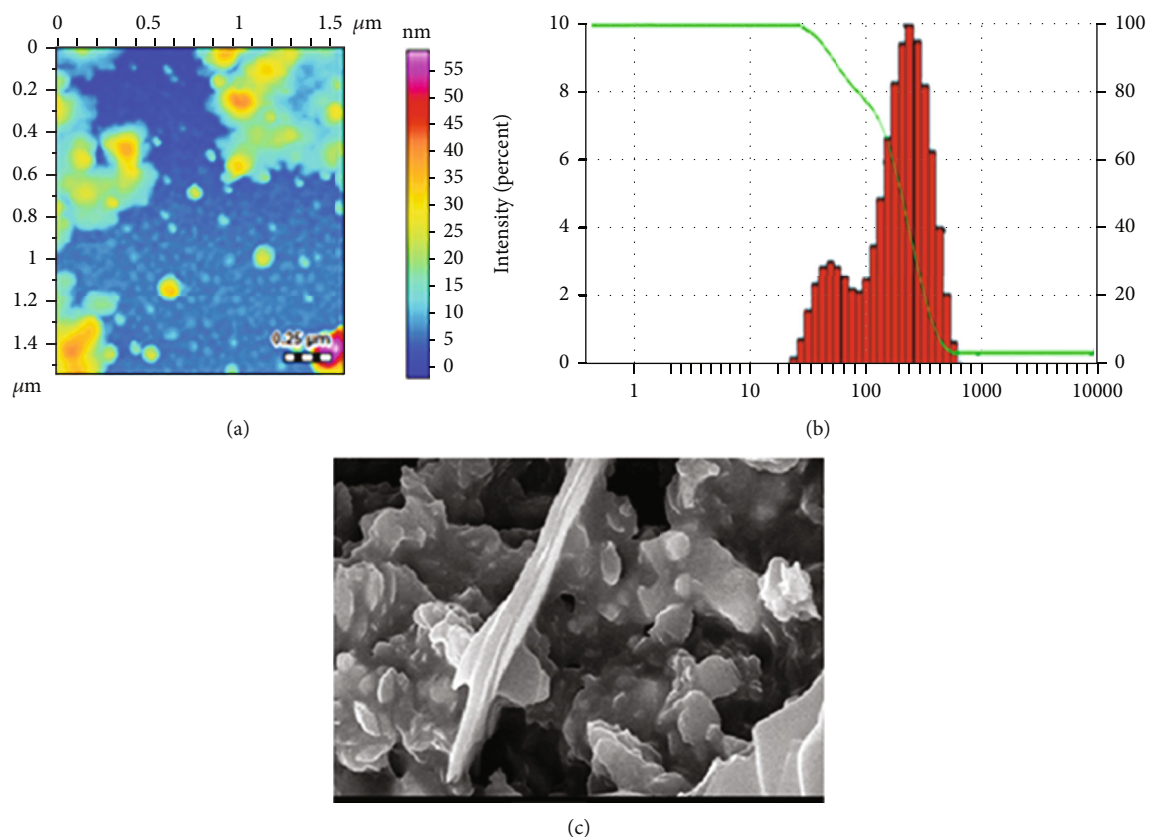
(a)

(b)

(c)

Figure 10: Change in surface morphology and size of ACR-AgNPs upon complex formation with Hg^{2+}. (a) AFM image of ACR-AgNPs-Hg^{2+}. (b) Change in particle size of ACR-AgNPs upon addition of Hg^{2+}. (c) SEM image of ACR-AgNPs-Hg^{2+}.
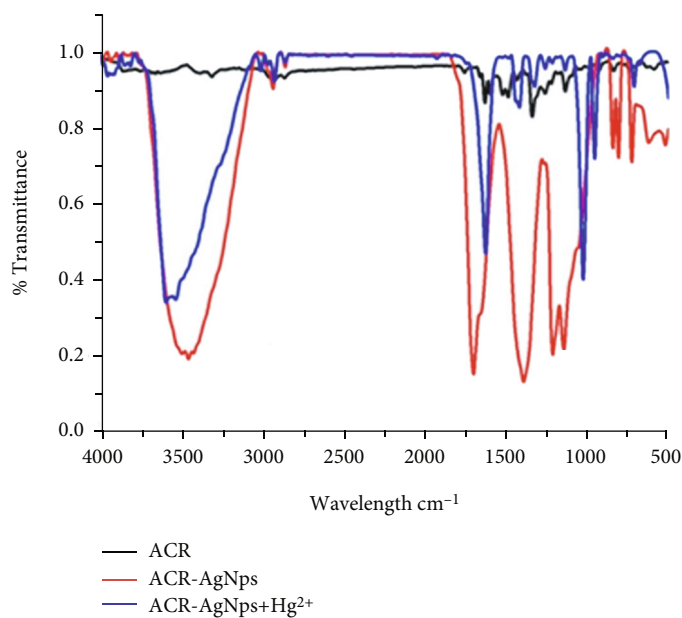

Figure 11: FTIR spectra of ACR, ACR-AgNPs, and ACR-AgNPs complex with Hg^{2+}.

constant in a pH range of 6-8 followed by a slight decrease in the range of pH 8-10. Furthermore, absorption intensity of the ACR-AgNPs complex with Hg^{2+} increased in the pH range of 10-14, which may be attributed to the enhancement of nucleophilic character of the donor atoms that facilitate hydrogen bonding. The results
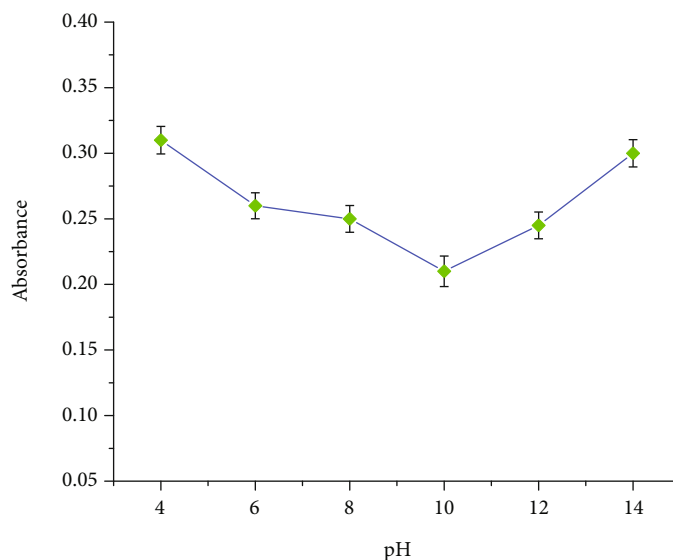
FIGURE 12: Stability of ACR-AgNPs complex with Hg^{2+} in the pH range of 1-14.

- ── ACR-AgNPs
- ── ACR-AgNPs+Hg (II) (D.I water)
- ── ACR-AgNPs+Hg (II) (Tap water)
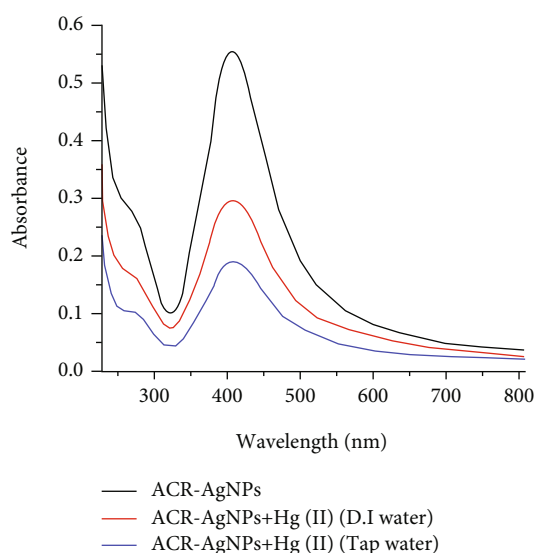
FIGURE 13: Application of ACR-AgNPs for the detection of Hg^{2+} in water sample.

TABLE 1: Results for the detection of Hg^{2+} in D.I water and tap water samples (mean ± SD, $n = 3$).

Samples	S. no	Added conc (μM)	Found conc (μM)	Recovery %	RSD %
D.I water	1	5	4.92 ± 0.23	98.4	1.6
	2	10	9.42 ± 0.13	94.2	1.4
	3	15	14.52 ± 0.09	96.8	2.5
Tap water	1	5	5.12 ± 0.08	102.4	2.3
	2	10	9.94 ± 0.11	99.4	0.9
	3	15	15.35 ± 0.13	102.3	1.4

indicate the effectiveness of the ACR-AgNP-based sensor for Hg^{2+} in the whole pH range.

3.6. Detection of Hg^{2+} in Tap Water. To assess the capacity of ACR-AgNPs as nanosensor for Hg^{2+} in water sample, two different Hg^{2+} solutions had been prepared in tap water and deionized water (collected from University of Karachi, Pakistan), respectively. It was noticed that the existence of electrolyte did not alter the absorption spectra and quenching observed by adding nanosensor in mercury (Hg^{2+} ions) solution made up in either deionized water or in laboratory tap water (Figure 13). Similar suppressions are recorded in the intensity of ACR-AgNPs after the addition of mercury in both deionized water and tap water. The percentage recovery of the spiked samples is in the range of 94.2-102.4 as depicted in Table 1. The results illustrate that ACR-AgNPs can be used as a nanosensor for the selective detection of Hg^{2+} in tap water sample.

3.7. Comparison with Other Studies. Up till now, several analytical methods are reported in the literature for the selective detection of Hg^{2+}. Most of the reported methods to detect the mercury (Hg^{2+} ions) in the biological system and aqueous media are difficult, tedious, and costly, Table 2. As can be seen in the Table that the limit of detection of the instrumental methods is lower compared to current method. However, these methods are based on the expensive instruments and time-consuming analysis protocols. The method proposed in this study is based on UV-visible spectroscopy which is easy, economical without any need of an expert. Furthermore, the material used for the synthesis of ACR-AgNPs is cheaper compared. The method is swift, on-spot, and works well for real samples (water samples and biological system).

TABLE 2: Comparison method for the detection of Hg^{2+} ions in tap water.

Techniques	Stabilizing agent	LOD	Reference
Fluorescence	DNA-scaffolded silver nanoclusters	$2.4 \times 10^{-5} \mu M$	[44]
Colorimetric sensing	Polymer matrix type chitosan matrix nanocomposite	$7.2 \times 10^{-3} \mu M$	[45]
Fluorescence	Tryptophan stabilized fluorescent Ag nanoclusters	$6.58 \times 10^{-7} \mu M$	[46]
Fluorometric turn-on and colorimetric detection	Poly(acrylic acid)-templated silver nanoclusters	$2 \times 10^{-3} \mu M$	[47]
Fluorescence	DNA/AgNC probe	$2.1 \times 10^{-3} \mu M$	[48]
Electrochemiluminescence	Oligonucleotide	$2 \times 10^{-2} \mu M$	[49]
UV-visible spectroscopy	Silver nanoparticles	$1.65 \mu M$	This study

4. Conclusion

Acridine-based conjugated silver nanoparticles showed effective and sensitive method for the detection of Hg^{2+} ions in the real water sample, and these nanoparticles also showed environmentally friendly behaviors. The addition of Hg^{2+} ions prepared in the tap water with solution of silver nanoparticles of nanosensor (ACR-AgNPs) presents a significance decrease in the absorption intensity. The silver nanoparticles of acridine showed selectivity even in the presence of different metals ions and also exhibit significant stability in acidic and basic environment. The presence of electrolyte does not show any disturbance on the absorption intensity of the conjugated silver nanoparticles of acridine. Therefore, these results illustrate that derivative of acridine can be used as an efficient sensors for the detection of environmental pollutants. The limit of detection of proposed sensor obtained from experimental data is $1.65 \mu M$. The percentage recovery of the spiked samples was in the range of 94.2–102.4%. All the above results show that the nanosensor could be used for the selective detection of mercury in the laboratory tap water and in biological samples.

Data Availability

All the data is presented in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Novel Application for Activated Carbon Pharmaceutical Formulation as an Efficient Adsorbent of Iron (III) from Contaminated Canned Food

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Additional medical indication has been realized for the active carbon commercialized in pharmaceutical stores for the first time. In this study, the active carbon was used to remove iron (III) from real canned food samples contaminated with iron by an adsorption process, in a new approach. Different parameters affecting the adsorption behavior of active carbon towards iron (III) have been optimized. Under the optimized conditions, namely, pH 4, 5 h contact time, 40 ppm iron initial concentration, and 2 g carbon, the study was applied to some of the real canned food samples. Black olive and green bean canned food samples containing relatively high iron levels of 698.0 and 1168 mg kg^{-1}, respectively, have been selected for this study due to their levels which were above the maximum permitted levels 48 mg kg^{-1} according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA). These samples were digested and treated, and their iron concentration was determined using ICP-AES under the specified optimum conditions. The removal percentage of the real samples was ~91%. In a novel approach, this study simulates the adsorption behavior of active carbon towards iron (III) in the human stomach when canned food with relatively high iron levels has been digested, since the pH value of the human stomach (~3) comes in good agreement with the adsorption optimized pH range (2-4). Moreover, the overall tolerability and safety of active carbon are worldwide known and traditionally documented. These application studies realize the applicability of utilizing active carbon commercially available in pharmaceutical stores to eliminate the toxicity of canned food contaminated by high levels of the investigated elements.

1. Introduction

The problems of the ecosystem are increasing with the development of technology. Heavy metal pollution is one of the main problems [1]. Toxic metal compounds coming from the earth's surface not only reach the earth's waters (seas, lakes, ponds, and reservoirs) but can also contaminate underground water in trace amounts by leaking from the soil after rain and snow. Therefore, the earth's waters may contain various toxic metals. Drinking water is obtained from springs which may be contaminated by various toxic metals [2, 3]. One of the most important problems is the accumulation of toxic metals in food structures. As a result of accu-

mulation, the concentrations of metals can be more than those in water and air. The contaminated food can cause poisoning in humans and animals. Although some heavy metals are necessary for the growth of plants, after certain concentrations, heavy metals become poisonous for plants, animals, and humans [4, 5].

Although the organic pollutants can be biodegraded, heavy metals do not degrade into harmless end products [6]. Iron is one of the major constituents of the lithosphere and comprises approximately 5% of it. It is routinely detected in municipal waste effluent, particularly in cities where iron and steel are manufactured. Iron readily complexes with sulphates in the sediments of many surface levels

stored in a fresh plastic bag after cooling and ready for digestion.

2.5. Sample Digestion. Digestion of food samples for iron metal analysis involved a high-performance microwave-assisted digestion using (Closed Vessel Acid Digestion-MARS System-CEM). In this case, portions of 0.5 g of dried samples were weighed and deposited directly onto the base of clean, dry Teflon microwave digestion vessels (Easy Prep vessel). A total of 10 mL of concentrated nitric acid HNO_3 (69%) were added to the investigated food samples that were sealed and digested via one-stage temperature ramping (ramped to 200°C for 25 minutes, held for 15 minutes). Two blank samples containing only nitric acid were prepared in the same way. The solution was allowed to cool, quantitatively transferred into a 100 mL volumetric flask, and then diluted to the mark with deionized water. After dilution, all digested sample solutions were cleared before analysis by ICP-AES.

2.6. Iron (III) Metal Analysis. The iron metal was analyzed using ICP-AES under optimized plasma conditions. Using the autosampler, the measured samples were nebulized downstream to the plasma, and the concentrations were automatically determined using the standard calibration graph. The system was adjusted to measure the samples in triplicate, and the relative standard deviation was automatically calculated. The RSD was <2%, and the correlation coefficient was >0.99998.

2.7. Batch Adsorption Experiments. To assess the removal capability of high levels of iron ions, the adsorption behavior of such metals has been performed under optimized conditions. In this study, iron (III) and carbon have been selected in this study as examples for contaminant and adsorbent, respectively.

The stock solution of iron (III) was prepared by dissolving an appropriate amount of salt (0.3 g) in (1000 mL) of deionized water. The different working solutions were properly prepared by dilution of appropriate aliquots of the stock solution using deionized water.

Based on its availability in local drug stores as well as its safety as a treatment of some diseases, the active carbon obtained from these stores has been used as an adsorbent material. The active carbon-based drug was properly grounded using a laboratory jar mill before using in-batch experiments.

To optimize the removal process, many parameters that affect the adsorption behavior of Fe (III) on carbon materials have been studied. These parameters include the effect of Fe (III) concentration, the effect of contact time, the effect of carbon weight, and the effect of pH.

2.7.1. The Effect of Initial Concentration of Iron (III) Ions on the Removal Percentage. The effect of the initial concentration of Fe (III) on its adsorption behavior on carbon has been investigated. A series of multistandard solutions (with concentrations 0.62, 2.42, 5.87, 18.52, 40.26, and 72.61 mg L^{-1}) was prepared and used in this study. The pH of each test solution was adjusted at the value of 4. To 50 mL of each solution, 1 g of carbon (2.8 g of drug formulation) was added, and the mixtures were shaken in a shaker at about 600 rpm for 5 hours. After that, the mixtures were filtered using filter paper. The filtrate (after shaking with carbon) and the multistandard solu-

tions (before shaking with carbon) were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.7.2. The Effect of Contact Time on the Removal of Iron (III) Ions. To determine the equilibrium time, the effect of contact time on the adsorption behavior of carbon towards iron (III) has been studied. In this study, a standard solution whose concentrations were 40 mg L^{-1} was prepared. To different aliquots of 50 mL of the standard, 1 g of carbon was individually added, and the mixtures were shaken in a shaker at about 600 rpm for 5, 15, 30, 60, 120, and 300 minutes. Each solution was then filtered using filter paper. The filtrate was analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The investigated contact time was selected in the range of 5 to 300 min to simulate the time that the food generally spends in the human stomach, since the food spends about 5 h in the human stomach [2].

2.7.3. Effect of Weight of Carbon on the Removal of Iron (III) Ions. The effect of adsorbent weight on the adsorption behavior of carbon towards iron (III) has been investigated. In this study, a standard solution containing (Fe^{3+}) ions whose concentrations were 40.97 mg L^{-1} was prepared. To different aliquots of 50 mL of standard solutions, 0.1, 0.5, 1, and 2 g of carbon were individually added. The different solutions were then shaken, filtered, and analyzed using the inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.7.4. Effect of pH on the Removal of Iron (III) Ions. The effect of pH of the test solutions on the removal percentage of iron (III) by carbon has been investigated. In this study, the pH values of the working solutions were adjusted using small quantities of concentrated nitric acid and sodium hydroxide. A series of standard solutions of iron (III) with a concentration of 40 mg L^{-1} was prepared at different pH values from 2 to 4. Then, to each solution, 1 g of carbon was added, and the solutions were shaken in the shaker for 5 hours. The solutions were then filtered and analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.8. Application. Under the optimized condition which extracted from the previous studies, the application of the removal of some investigated metal ions from some real canned food samples has been assessed. The optimum conditions were as follows: pH of 4, contact time of 5 hours, initial concentration of iron (III) of 40 mg L^{-1}, and weight of carbon adsorbent of 2 g (5.6 g of the drug). Iron (III) has been selected as an example for this study based on its high concentrations found in some of the canned food samples in comparison to their respective fresh food samples in our previous work [16]. The canned food samples underinvestigated in this study were treated similarly to the above-mentioned studies and under the optimized conditions. Thus, the removal percentage was also calculated in the same manner.

In all studies, the percent removal of iron (III) ions was calculated using the following equation [20]:

$$R\% = \frac{C_o - C}{C_o} \times 100, \qquad (1)$$

where C_o is the initial iron (III) ion concentration (before the addition of carbon) of the test solution, $mg\,L^{-1}$, C is the final iron (III) ion concentration (after the addition of carbon) of the test solution, $mg\,L^{-1}$, and $R\%$ is the removal percentage.

3. Results and Discussion

In a novel approach, the adsorption behavior of iron (III) on active carbon present in some local drugs as an adsorbent has been assessed for the first time in this study. Systematic studies of the adsorption of ferric ions on active carbon under different experimental conditions have been carried out to understand the nature of the adsorption process. It is worth mentioning here that iron has been selected in this study as an example of the investigated metals determined in the studied canned foodstuffs due to their levels being above the maximum permitted levels in about 60% of the investigated canned food samples [16]. However, the investigated drug containing about 60% charcoal has been selected in this study as a sorbent based on its availability in local drug stores. It has been used safely for a long time as a laxative and relieves gas pain by a lot of household people. Moreover, charcoal, which represents a high percentage of the drug, has been used in the removal of heavy and toxic metals from different media in environmental, industrial, and food strategies [21]. Activated carbons are widely used as adsorbents due to their high adsorption capacity, high surface area, porous structure, and high degree of surface, respectively. The chemical nature and pore structure of carbon usually determine the sorption activity [22, 23]. More specifically, the commercialized activated carbon was produced in different formulations based on its application. These revealed BET-specific surface values ranging from 500 to 2000 m^2/g. In addition, the superactivated carbons provided surfaces area higher than 3000 m^2/g. The macro-, meso-, and micropore volumes of the activated carbon magnitude are between 0.5 and 2.5 m^2/g [24–26]. Moreover, the nature of adsorption and its kinetics thermodynamics of versatile species on activated carbon have been reported [24–26]. Instead, this work oriented and reported a new application of the commercial activated carbon in a very promising and novel approach.

3.1. Effect of Carbon Weight on the Removal of Iron (III) Ions. The effect of carbon weight as a sorbent on the removal percentage of iron (III) from aqueous media has been assessed. The experiments were carried out with 0.1, 0.5, 1, and 2 g of carbon, which is equivalent to 0.3, 1.4, 2.8, and 5.6 g of drug tablets, respectively. Appropriate amounts of iron (III) chloride were individually used to prepare standard solutions of iron (III) of 40.97 $mg\,L^{-1}$ concentration. The mixtures were vigorously shaken for 5 hours and then filtered. The concentrations of the standard iron solutions

were determined directly before and after shaking using ICP-AES under the optimized condition mentioned in the experimental section. The removal percentages were then calculated using Equation (1). The results obtained are presented in (Figure 1). As can be seen, the removal percentage increases with increasing the weight of active carbon sorbent until it reaches a steady-state value. Increasing the removal percentage of iron (III) with increasing the weight of active carbon is attributed to the increase of the adsorption capacity and hence the active sites of the sorbent, which would increase the adsorption of the investigated metal ion [4, 6, 7].

Inspection of the data obtained showed that the maximum removal percentage was obtained for Fe^{3+} ions, which is nearly equal to 94.28%, and the minimum removal was obtained for Fe^{3+} at 38.44%. The removal percentage significantly increases as the weight of sorbents increases. A carbon weight of 2 g which gives the maximum removal percentage has been selected in the rest of the experiments and in the application, as well.

3.2. Effect of Contact Time on the Removal of Iron (III) Ions. The effect of contact time on the adsorption behavior of carbon towards iron (III) has been examined. In this study, amounts of 1 g of carbon (2.8 g of drug) were added to aliquots of 50 mL of standard iron (III) of concentration 40 $mg\,L^{-1}$. The mixtures were vigorously shaken for different time intervals and then filtered. The concentrations of the standard iron solutions were also determined directly before and after shaking using ICP-AES under the optimized condition mentioned in the experimental section. The removal percentages were then calculated using Equation (1). The investigated contact time was selected in the range of 5 to 300 min to simulate the time that the food generally spends in the human stomach, since the food spends about 5 h in the human stomach [2]. As shown in Figure 2, the removal percentage of carbon for iron (III) increases as the contact time increases, the maximum removal value for iron (III) was obtained with a contact time of 5 hours, and the value was equal to 72.02%. Thus, the contact time of 5 hours was used for the rest of the experiments.

3.3. Effect of pH on the Removal of Iron (III) Ions. The effect of pH is one of the most important parameters controlling the uptake of heavy metals from aqueous solutions [20]. The effect of pH of the test solution on the adsorption behavior of carbon presents in the drug towards iron (III) has been investigated. In this study, amounts of 1 g of carbon (2.8 g of drug) were added to aliquots of 50 mL of standard iron (III) solutions of a concentration of 40 $mg\,L^{-1}$. The pH of the solutions was adjusted to different values using small aliquots of concentrated nitric acid and sodium hydroxide solutions. As mentioned above, the concentrations of iron (III) in the different solutions were properly determined directly before and after shaking the mixture for 5 hours. The results obtained presented in Figure 3 show that the removal percentage of iron (III) was independent of the pH of the test solution in the investigated pH range (2-4), which indicates that the maximum adsorption affinities occur in moderately and slightly acidic medium. Experiments with higher pH values were
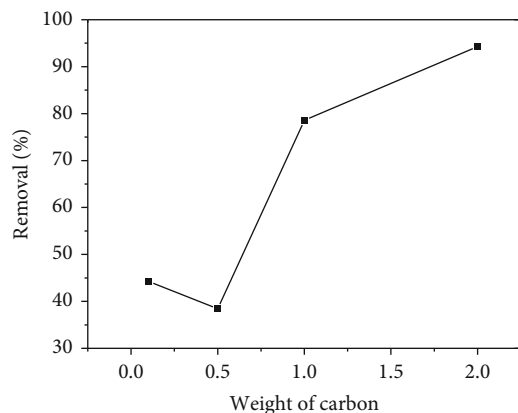
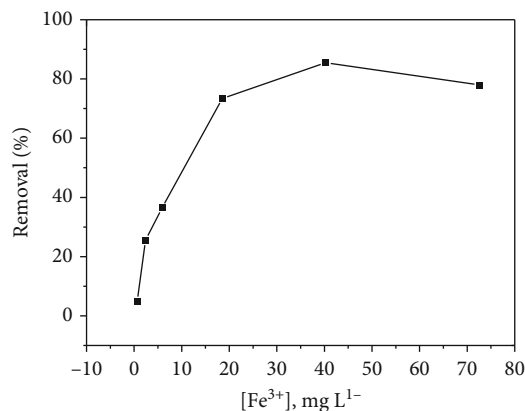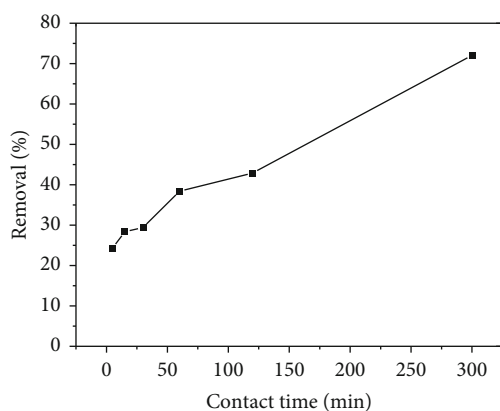FIGURE 1: Effect of the carbon weight on the removal of iron (III).

FIGURE 2: Effect of the contact time on the removal of iron (III).
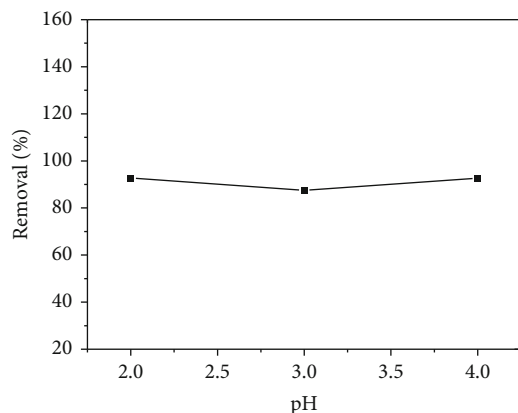

FIGURE 3: Effect of the pH on the removal of iron (III).

avoided to prevent the precipitation of iron (III) as iron hydroxide. This independent pH range is almost fortunately the pH range of the human stomach [27].

Indeed, the adsorption behavior study of carbon present in drugs on the removal of relatively high concentrations of iron (III) simulates its adsorption behavior to such metals in

FIGURE 4: Effect of the initial concentration of iron (III).

the human stomach when poisoned with canned food containing high levels of the investigated elements.

3.4. Effect of Initial Concentration of Iron (III) Ions on the Removal Percentage. The effect of initial iron (III) concentration on the adsorption behavior of carbon present in the drug has been investigated. In this study, amounts of 1 g of carbon (2.8 g of drug) were added to aliquots of 50 mL of standard iron (III) solutions of different concentrations. After shaking vigorously for 5 hours, the solutions were separated, and the iron concentrations have been also determined. The variation of the removal percentage with the change in initial concentration of iron (III) ions is shown in Figure 4. The removal percentage of iron (III) increases as the initial concentrations increase up to $40.26 \, mg \, L^{-1}$, then decreased as the initial concentration increases. As can be seen, at a constant adsorbent dose (1 g), and a constant time (5 h), the removal (%) of iron (III) increases due to the increase in the sorbent capacity until the steady state reaches the maximum capacity. However, at a higher concentration, the removal (%) gradually decreases with increasing metal concentration which is attributed to the numbers of iron (III) ions relatively higher than the available active sites of the sorbent. Thus, after saturation, the initial concentration of the investigated metal ion has a restricting effect on the removal capacity whilst simultaneously the sorbent media has a limited number of active sites [4, 6, 7].

3.5. Application on the Adsorption Study. Different parameters affecting the adsorption behavior of carbon towards iron (III) have been optimized as previously discussed. These optimized conditions were found 2 g of carbon weight (5.6 g of drug), 5 hours contact time, pH range of 2-4, and $40 \, mg \, L^{-1}$ initial iron concentration. Under the optimized conditions, the adsorption behavior study of carbon towards iron (III) has been applied to real food samples. Based on our previous work [16], black olive and green bean canned food samples containing relatively high iron levels of 698.0 and $1168 \, mg \, kg^{-1}$, respectively, have been selected for this study. These samples were digested using a microwave system and treated, and their iron concentration was determined as described above using ICP-AES under the

TABLE 1: Application studies on real canned food samples.

Sample type	Food type	The level of iron (III) pretreatment (mg kg^{-1})	The level of iron (III) after treatment (mg kg^{-1})	Removal (%)
Canned food	Black olives	698.0	66.00	90.5
	Green beans	1168	100.8	91.4

specified optimum conditions. The results obtained are presented in Table 1. The removal percentage of the real samples was ~91%. This study simulates, for the first time, the adsorption behavior of carbon towards iron (III) in the human stomach when canned food with relatively high iron levels has been digested, since the pH value of the human stomach (~3) comes in good agreement with the adsorption optimized pH range (2-4). Moreover, the overall tolerability and safety of active carbon drugs are worldwide known and traditionally documented [28–30]. These application studies realize the applicability of utilizing the active carbon drug to eliminate the toxicity of canned food contaminated by high levels of the investigated elements.

4. Conclusions

In a new and very promising application of drugs containing active carbon, the adsorption behavior of contaminant iron (III) on such drugs has been reported. The study simulates the behavior of the tested contaminant inside the human stomach to avoid the toxicity of the tested species in case of chemical poisoning, since the concentration of iron metal has been detected in black olives and green beans using ICP-AES under optimum conditions and after digestion using a closed vessel microwave digestion system in our previous work. The data obtained showed that the iron metal has exceeded the international tolerance levels in some investigated samples which were collected from the local markets of the western province of Saudi Arabia. In this context, the parameters affecting the adsorption behavior of carbon present in the active carbon drugs towards iron (III) have been investigated and optimized. Under these optimized conditions, namely, pH 4, 5 hours contact time, 40 mg L^{-1} iron initial concentration, and 2 g carbon (5.6 g of the drug), the study was applied to the real canned food samples (black olives and green beans) contaminated by relatively high concentrations of iron after digestion using microwave system. The results showed that there was about ~91% removal of iron (III) from the investigated real canned food samples. This study simulates the elimination of toxicity in the human stomach that comes from the digestion of foodstuffs contaminated by the investigated toxic heavy metal, since the pH value of the human stomach (~3) comes in good agreement with the adsorption optimized pH range (2-4). Moreover, the overall tolerability and safety of active carbon-based drugs are worldwide known and traditionally documented.

Data Availability

All data supporting this work are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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