

ARSENIC (V) REMOVAL FROM MINE DRAINAGE BY BIOACTIVE COMPOUNDS IN FRUIT WASTES

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Abstract. Acid mine drainage (AMD), is the largest environmental problem facing the mining industry today which is caused by the biological oxidation of sulfidic materials, frequently contains arsenic in the form of arsenite: As(III), and/or arsenate: As(V). The current study is conducted toward the removal of arsenic from synthetic AMD by raising the pH of the solution and utilizing bioactive compounds in fruits waste/ byproducts from food industry process by adsorbing arsenic to recover the contaminant. Arsenite is likely to be quickly converted to arsenate, the more thermodynamically stable form. The current study was designed for utilization of sour lemon peel (SLP) and Grape pomace (GP) as less expensive and much frequently available food byproducts due to prospective Arsenic bio-sorption capacity. For cleaning up Contaminated mining sites soil and the in order to ascertain its adsorbing potential and probable bio-adsorption capability of this fruit wastes, in August and September 2022 composite soil samples were collected within the Anguran (Zanjan Province), mines and simulated the conditions of soils in the contaminated sulfidic lands As(NO₃)₃ was added. The soils were mixed thoroughly and all samples stored at a greenhouse in Tehran County. Arsenic contents in all samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). Results revealed that all As oxidation forms in treated soil were lower content significantly ($p < 0.03$) than untreated soil samples after a few days and removal of As(V) has done more than other form of As(III), ($p < 0.01$). The removing process of Arsenic from soil reached more than 99% in 20 days in pH of soil 3.6 and in presence of 5% SLP & 2% GP. Current study provides an optimistic alternative remediation of arsenic contaminated soils emphases on low cost and environmental treatment.

Keywords: Bio-adsorption process, clean-up soil, fruit waste, mine drainage.

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1. Introduction

Acid mine drainage (AMD) is one of the major environmental problem in many sulfidic mines, mainly by low pH resulting in an acidic domain and as a consequence, high concentrations of several heavy metals (Luo *et al.*, 2020). Among heavy metals Arsenic, Mercury, Lead, Chromium and Cadmium have been the most common and major induced human poisonings (Mokhtarzadeh *et al.*, 2021; Ziarati *et al.*, 2020; Hochwimmer *et al.*, 2020). Unlike organic pollutants, toxic heavy metals and metalloids can not be broken down

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complexly and are therefore long term problems, though are often simultaneously associated with organic and other pollutants. A major concern then is their accumulate along the food chain to high levels in body tissues of animals or human that may damage DNA with carcinogenic effects through their mutagenic ability (Mustafa *et al.*, 2023; Alarcón-Herrera *et al.*, 2013). Arsenic has had a long historical use by humans and its adverse health effects have been known for mellenia (Yunus *et al.*, 2019; Khan *et al.*, 2022). It has been used in both ancient and modern medicine and abused as a stimulant in people and animals such as in race horse doping with potassic arsenic. Such malpractice occurs alongside metals including cadmium, mercury, bismuth, gold or platinum chlorides use. Significantly, arsenic appears synergistically to potentiate the adverse health impacts of these heavy metals. Arsenic (As) is it highly toxic and carcinogenic metalloid (Jaishankar *et al.*, 2014). Exposure to arsenic in humans is universal at some level of which there are no known safe standards, especially in foods, though nominal set by the WHO at 10ppb As in drinking waters (Ziarati *et al.*, 2019; Ziarati *et al.*, 2017). Widespread naturally, arsenic concentrates in certain geological formations such as sedimentary basins and their ground waters having emanated regionally from mineralized country rocks. Arsenic is widespread in the industrial and agricultural sectors, including animal feed used to control pathogens, weeds and pest, both aquatic and terrestrial. Arsenic occurs in low to high levels in many every day products, food such as rice, tobacco, drinking waters and can attain very high levels in natural geological environments, including metal deposits, coal deposit and under the right geogenic conditions, sedimentary basins and their groundwaters. Given the synergistic effects of arsenic, there are likely no safe limits to arsenic exposure (Raju, 2022; Bakhshalizadeh *et al.*, 2023).

Arsenic has no know essential functions in humans, though some microbes utilize arsenic in chemotropic reactions. Many oxidoreductases, critical to normal biological functions, are sequestered by bacteria when confronted with xenobiotics such as arsenic in terms of detoxification attempts via metalloid species transformation and mobilization. Class EC 1.20 reductases for instance act on phosphorous or arsenic, arsenates being able to participate in competitive exchange with phosphates, one of the toxic modalities of arsenic in humans (Sánchez-Riego *et al.*, 2014). Excess environmental xenobiotics can also be used to derive energy by microbes via oxidation of iron, sulfur, manganese and arsenic. Alternatively, dissimilatory reduction of metals by some microorganisms also enables them to use metals as a terminal electron acceptor for anaerobic respiration (and producing hydrogen sulphide). Oxyanions of arsenic may be used in microbial anaerobic respiration as terminal electron acceptors, also well known for oxyanions of chromium, selenium and uranium (Wang & Zhao, 2009; Dong *et al.*, 2023). It is important to understand these reactions for capture and release of arsenic in the environment.

Industrial and agricultural arsenic liquids drain into and solids enter soil and waterways through indiscriminate discharge (Jafari Moghadam *et al.*, 2016). Widespread use of **Paris green, a copper acetoarsenite** pesticide adds to this contamination milieu along with sodium arsenite, lead arsenate, calcium arsenate, magnesium arsenate and zinc arsenate, zinc arsenite in aquatic weed control as well dusted on orchards, crops and produce such as tobacco for pest control (Dong *et al.*, 2023). Substantive contamination to residential soils, urban market garden soils, urban and agricultural groundwater's have occurred worldwide from these anthropogenic practices, but also from natural groundwater's. Many of these ground waters are used for potable drinking waters as well

agriculture. Very heavy pollution of this type has for example occurred in residential areas such as Calcutta, India (Haque & Singh, 2017).

Such arsenic sources are easily absorbed, and accumulate in aquatic and terrestrial biota, magnifying up the food chain. Excessive manure fertilizer is thereby another source of arsenic pollution in soils and waterways, as they are for toxic cadmium. This encouraged many countries to ban the practice, but it is still common. Inadequately processed grey water recycling, common now due to increasing aridity, adds heavy metal and metalloids such as arsenic if not processed to remove it. Poultry manure can lead to high arsenic concentration in animals and humans since their feedstocks can be fortified with arsenic to keep pathogens down (Muhammad *et al.*, 2022). The practice of preserving herbal formulations with arsenic is still practiced in some quarters. Tattoo media is also a source of metalloids, including arsenic, environmentally assessed in this study BC-arsenic urine study.

In countries such as India and within Asia such as China, generally where sedimentary basin may have high arsenic, arsenic ingress to rice is to be considered seriously, along with cadmium (Jia *et al.*, 2006; Sun *et al.*, 2013). Particularly in knowledge, arsenic may potentiate the effects of cadmium synergistically. For this reason, rice was considered in this BC-arsenic urine study, as one of the potential environmental factors as it was in our previous study of Cadmium in breast cancer (Ziarati *et al.*, 2020). Rice in Iran, as in many developing countries has become a staple food. With approximately $2.35 \times 10^{12} \text{ M}^2$ of arable land worldwide considered polluted, this becomes a very serious consideration for Medical Geology with potential long term chronic exposure of metal (loids), yet unknown globally, together with known acute toxicities, such as **Itai-Itai** disease in Japanese women (Lahiji *et al.*, 2016; Gholizadeh & Ziarati, 2016). Both our BC arsenic and BC cadmium studies considered cheap Indian rice imports as problematic and recommended close controls on food quality generally with international standards that are lax in terms of food import and exports control. Medical Geology mapping of the glob may be another solution (Ziarati *et al.*, 2020; Hochwimmer *et al.*, 2020). Geology and watering practice growing rice and agricultural soil contamination generally, augmented from air pollution surrounding large industrial cities is of growing concern for metalloid build up in soil, near surface ground water quality and crops, particularly within replete market and urbane gardens close to industrial centers (Motaghi & Ziarati, 2016; Razafsha & Ziarati, 2016).

Geogenically, remobilized arsenic from geologically mineralized terrains entering sedentary basin strata compound issues of arsenic mobility within polluted ground waters is one of the greatest Medical Geology concerns (Hochwimmer *et al.*, 2020; Ziarati & Hochwimmer, 2018). Such mineralized geologies add substantive metals to soil and waterways and are highly problematic for livestock and human drinking water, market gardens and agriculture. This is especially so in dry and wet season alternation of oxidation and reduction reactions seasonally, magnifying arsenic levels and mobility along with ingress to biota, food chains and drinking waters. Medical Geology aims to address these issues, considered in the review section (Ziarati *et al.*, 2016; Ziarati *et al.*, 2015; Albaji *et al.*, 2013).

Natural ground waters leaching arsenical minerals from mineralized geologies are the single largest source of arsenic impacting humans and animal health from drinking and agricultural waters. Arsenic laden drinking waters set by the WHO standard of 10ug/L (Wang & Zhao, 2009), affects many worldwide with acute and chronic exposure. Worldwide, such aquifers occur within Bangladesh, West Bengal and other parts of India, worst affected. Other regions affected are within China, Taiwan, Hungary, Romania,

Mexico USA, Thailand and Vietnam (Herath *et al.*, 2016). However, no consensus exists on how wide scale the global problem is, other than the scope of arsenic pollution is extremely large, known sources afflicting more than 100 million people. The afflicted areas of West Bengal typify the problems associated with poor demographic regions to deal with the issues (Xu *et al.*, 2021). This has led to low adoption of remediation strategies that may work in a laboratory setting but not in a practice sense for cultural reasons, including lacking the following: awareness; a 'sense of belonging'; willingness to take responsibility; safe sanitary disposal of arsenical sludge's; provision of staff operating salary and plants chemicals (Hu *et al.*, 2023; Wang & Ali, 2023). In India-West Bengal and Bangladesh, despite the four decades since the arsenic waters issues were noted exposures have worsened, not improved! Especially in the rural areas, a plethora of new wells some 10 million now has been drilled. Remediation and technologies educational strategies are therefore critical in these regions.

Microbial reduction mechanisms may be decoupled from respiration for other purposes, such as imparting metal resistance, for instance Hg (II) to Hg (0). The later has a very low solubility produce in water, important environmentally, as opposed to say methyl mercury. Others of environmental importance include Cr (VI) to Cr (III), reduction of Se (VI) to Se (0) and U (VI) to U (IV). These geo-microbiological and ancillary biochemical reactions are important in the mobility and toxicity of such species, including arsenic species, but here oxidation of As (III) to less toxic As (V) arsenates, or conversion to arsenopyrite or adsorption of such arsenates by oxy hydroxide of iron for arsenic stability is important environmentally (Rahman *et al.*, 2021; Sahu *et al.*, 2021). Microbes play a critical role in arsenic species transformations, geogenic mobility, toxic species transformation and environmental impacts of metal (loids), including arsenic. The biogeochemical mobilization of arsenic studied in concert with sulphides in acidic environments and neutral to slightly alkaline environments (Ziarati *et al.*, 2020; Ziarati *et al.*, 2019).

Recently scientific approach has been diverted towards the biomaterials which are byproducts or the wastes from large scale of agricultural waste materials or even vegetable and food processing wastes (Fatahi *et al.*, 2020; Ziarati *et al.*, 2021; Shirkhan *et al.*, 2022; Ziarati *et al.*, 2022).

Bioactive compounds outlying from fruits and vegetables wastes or by-products mainly include polyphenols, flavanols, tannins, flavonoids, vitamins (mainly A and E), essential and trace elements, minerals, fatty acids, volatiles, anthocyanins and pigments (Ben-Othman *et al.*, 2020; Ziarati *et al.*, 2020). The potential of food and agricultural waste to remove heavy metals from contaminated water and soil was conducted on the basis of open scientific sources (Ziarati *et al.*, 2019). From the patents and even convenient research, survey data, it is noticeable that a good deal of the fruits' wastes / by-products from fruit industry stands up after pressing the juice or after producing value-added products. Non-edible parts of fruits such as peels or skin portion and twigs frequently accommodate much higher quantity of bioactive compounds when compared to the edible parts (Gorinstein *et al.*, 2001; Gorinstein *et al.*, 2001; Ziarati *et al.*, 2021).

The current study is conducted toward the removal of arsenic from synthetic AMD by sustainable utilization of fruit wastes and by-products: Sour lemon peel (SLP) and Grape Pomace (GP) as available, low costs wastes by raising the pH of the solution and due to referring to previous studies by authors.

2. Material and methods

Soil Sampling

In August and September 2022, composite soil samples were collected within the Anguran in Zanjan Province 36°34'42"N 47°38'48"E mines and simulated the conditions of soils in the contaminated sulfidic lands. A total of 98 soil samples were collected randomly from the study area for bio-remediation investigation. All soil samples, mixed thoroughly, then 0.05 mole $\text{As}(\text{NO}_3)_3$ was added to the samples.

Bio-sorbent Sampling

There are two types of lime in English are two different names but are called in Farsi on both Lime: 1. Lime Green Shirazi (in English: Lime), 2. Lemon yellow Jahromy (in English: Lemon). The two lemons both in terms of appearance and color, and the flavor are completely different (Razafsha *et al.*, 2016). Native or original Sour lemon (*Citrus tamurana* and *Citrus Latifolia*) and Persian lime or "lemon Shiraz" RG family, which is the hybrid plant from two species: *Citrus aurantifolia* and Citrus Limon or *C. medica* peels was collected from 10 markets in Tehran-Iran. Yellow peels were chosen as adsorbent material. The fruits were washed extensively under tap water to remove adhering dirt, rinsed with de-ionized water, cut into small spices by small clean cutter and naturally dried in sunlight. Dried peel was grounded using a clean electric mixer, sieved through (Retsch GmbH & CoKG, Germany) mesh size (250 μm) to retain fine particles. To reduce enzymatic browning, the peels were then dipped in a 1% (w/v) citric acid solution for 10 min, drained and dried in an oven at 60°C for 24 hours and homogenized in a blender to utilize in adsorption experiments.

Soil profile characteristics were observed and recorded by a packet penetrometer (CI-700A, soil Test Inc., USA). Soil samples were mixed, homogenized and separated into three parts, 1/3 of each samples was air-dried and pass through a 2 mm sieve in order to determine p and k content, pH and electrical conductivity and particle-size distribution. The other 2/3 was passed through a 2 mm sieve without drying and 1/3 of it used to determine heavy metals concentration by ICP-OE, after digestion with aqua-regien. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines (Ziarati *et al.*, 2021; Fatahi *et al.*, 2020; Tavakoli-Hosseiniabady *et al.*, 2018; APHA, 2006).

All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid for 24hrs, cleaned thoroughly with distilled water and dried in such a manner to ensure that any contamination does not occur. Standard solutions contain 1000 mg L^{-1} arsenic (ICP Standard, Merck and Darmstadt, Germany) was used to arrange the calibration curve and reference solutions. All the solutions were prepared with Distilled-deionized water The residual acidity of the digests was determined by acid–base titration using sodium hydroxide solution standardized with potassium hydrogen phthalate. Set the hydride generating by utilizing hydrochloric acid solution (2 mol / L) (HCl, Merck: 37%), sodium borohydride (NaBH_4 tablets, purity > 97 %) at 1.5% in 0.2% NaOH (sigma Aldrich, Extra Pure). The HNO_3 , HCl and H_3PO_4 (Merck, analytical grade) concentrated acids used were diluted with ultrapure water to prepare the solutions for analysis. The reference material: (National Institute of Standards and Technology, NIST RM 8704), were used as reference for some tests. Real sample of complex mining waste, collected in a deposit of mining,

was used in the multivariate optimization and the recovery studies (Paula *et al.*, 2012; Ziarati *et al.*, 2013; Mirmohammad-Makki & Ziarati, 2014; Alimardan *et al.*, 2016).

The USEPA method was used to acid digest soil samples and metals present in the samples were detected using ICP-OES (EPA, 2014; Gazulla *et al.*, 2022). The heating program at 1800 W of applied power was conducted in two steps: at first stage to reach 140 °C and 5 min at 140 °C and 2nd step to reach 180 °C and 10 min at 180 °C. Subsequently, a ventilation step of 40 min was included to the heating program for cooling the samples. All studied samples cooled down till reached to between 20 and 25 °C (room temperature). The digestion procedure was monitored precisely (Amaral *et al.*, 2016; Prawira *et al.*, 2020).

The extracts were analyzed in an inductively coupled plasma optical emission spectrometer (ICP OES) model: Optima DV 7000 (Perkin Elmer instruments, Shelton, USA) with hydride generation. Single method-controlled dual viewing of the plasma delivers the widest working range possible, providing the lowest detection limits and the greatest concentration range in a single system. The operational conditions are introduced in table 1.

Table 1. Operational conditions of ICP OES.

Parameter	
Auxiliary gas flow rate (L min ⁻¹)	0.2
Plasma gas flow rate (L min ⁻¹)	15
Injector tube diameter (mm)	2.0
View	Axial
Interface	Shear gas
Applied power (kW)	1.3
Nebulization gas flow rate (L min ⁻¹)	0.6

Statistical Methods

All the data were analyzed using the SPSS 20 statistical software for analysis of variance using ANOVA and Duncan's least significant difference (LSD at $p < 0.05$) for statistical significance. Three duplicates with a replicate were considered in this research, and data was reported as the mean \pm standard error of the mean. The values reported here are means of five values. Each sample data was the mean of five subsamples. A p value of 0.05 or less was considered as statistically significant.

3. Results and discussion

Chemical extraction of the soil profile before adding specified amounts of Arsenic (III) Nitrate is shown in the table 1. Each data represents the mean of 3 replicates.

Table 2. Chemical characteristics of the soil profile at the studied samples (before adding chemical substances and pre-treatment by bio-adsorbents).

Layer (depth cm)	pH (H ₂ O)	Electrical conductivity dS/cm 1:1	NO ₃ -N mg/kg DW	NH ₄ -N mg/kg DW
1 (0-10)	6.3	0.39	62.7	8.84
2 (10-20)	6.5	0.20	32.9	8.22
3(20-35)	6.7	0.31	26.1	7.71

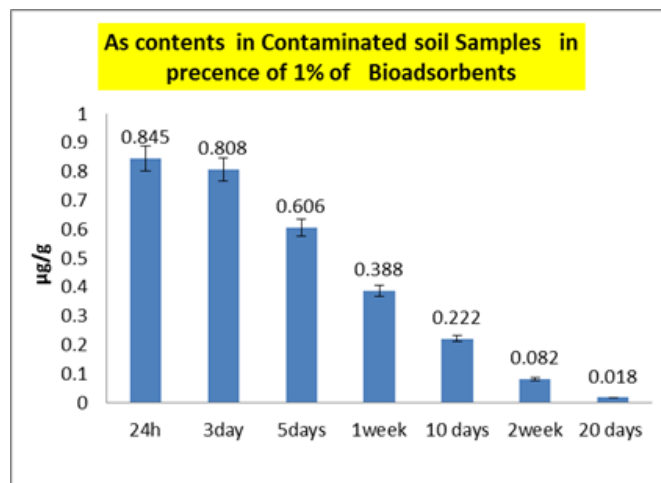


Figure 1. Effect of contact time on the removal concentration of Arsenic ($\mu\text{g/g} \pm \text{SE}$) from contaminated soils by 1% Gp & 1% SLP bio-adsorbent doses, Initial concentration of As Ions in Untreated samples = $1.109 \mu\text{g/g}$, temperature = $25 \pm 2^\circ\text{C}$, GP= Grape Pomace and SLP= Sour Lemon Peel wastes.

The effect of time in the constant concentrations/ percentage in dry weight of adsorbent on the removal of As ions by bio-mass of fruit wastes is depicted in figure 1, for adsorbent dose of 1% w/w of SLP & GP wastes. The results revealed the increasing of adsorbent capacities directly significantly affected the potentially increased time after 5 days of As removal from studied soils ($p < 0.003$) which means that treated by chelation agents can be boosted by the increase of the potential of bio-absorbent, removal ability of toxic heavy metal: Arsenic by biomass of Fruit Juice powdered byproducts/ wastes. After 20 days of treatment about % 98 of Arsenic removed from contaminated soil samples ($p < 0.001$).

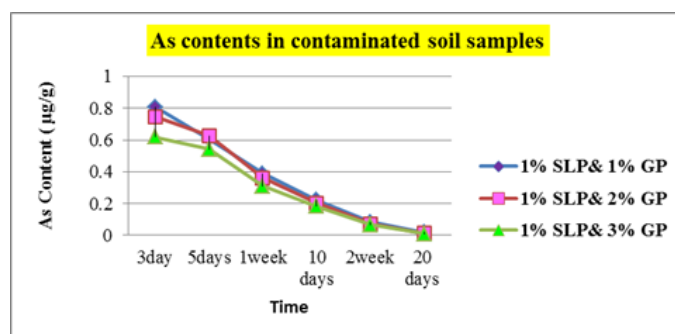


Figure 2. Effect of contact time on the removal of Arsenic from contaminated soils by different bio-adsorbent doses of GPomace and initial dose of 1% (W/W) SLP, Initial concentration of As Ions in Untreated samples = $1.109 \mu\text{g/g}$, temperature = $25 \pm 2^\circ\text{C}$, GP= Grape Pomace & SLP= Sour Lemon Peel wastes.

After 3, 5, 7, 10, 14 and 20 days of study in samples treated by 1% SLP and 2% GP mass as natural bio-adsorbent the As content reported: 0.7453 ± 0.003 , 0.6264 ± 0.002 , 0.3617 ± 0.002 , 0.2021 ± 0.003 , 0.0716 ± 0.001 and 0.011 ± 0.001 ($\mu\text{g/g DW} \pm \text{SE}$) respectively that proved that biomass of adsorbent can reduce toxic heavy metal mainly in the first days of treating. In the same trend, the decreasing contents of Arsenic can be presented by the results. After 3, 5, 7, 10, 14 and 20 days of study in samples treated by 1% SLP and 3 % GP mass as natural bio-adsorbent the As content reported: 0.6182

± 0.004 , 0.5408 ± 0.003 , 0.3112 ± 0.003 , 0.1837 ± 0.003 , 0.0676 ± 0.001 and 0.009 ± 0.001 ($\mu\text{g/g DW} \pm \text{SE}$). Obviously, by adding more content and percentage of biomass adsorbents, the potential of removal process increasing significantly. Citrus by-products represent a great potential for use as substrates in biotechnological processes.

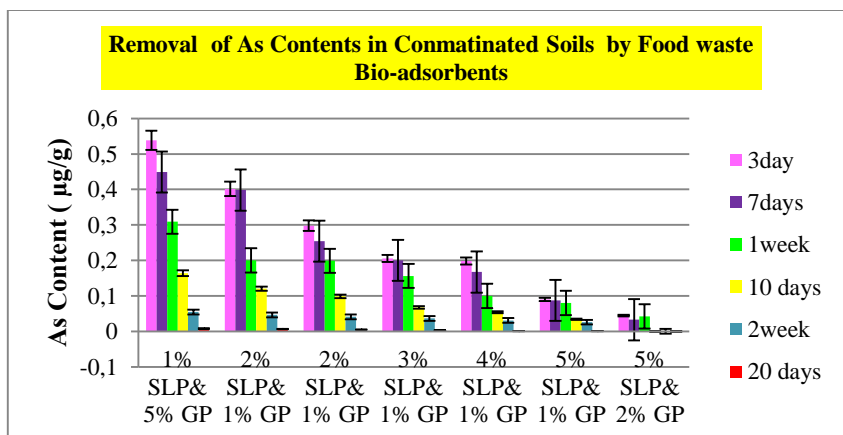


Figure 3. Effect of contact time on the removal of Arsenic contents ($\mu\text{g/g} \pm \text{SE}$) from contaminated soils by different contents of bio-adsorbent doses, Initial concentration of As Ions in Untreated samples = $1.109 \mu\text{g/g}$, temperature = $25 \pm 2^\circ\text{C}$, *GP= Grape Pomace and SLP= Sour Lemon Peel wastes

Results in figure 2, revealed significant difference in As up-taking by bio-adsorbent after 3, 5, 7, 10, 14 and 20 days in presence different doses of bio-mass. The data showed that time as an efficient factor (days) have significant differentiate ($p < 0.01$) and the potential of taking up As was increased significantly not only between the first day of study but also the trend is courteously effective by 20 days study and statistic state ($p < 0.001^{***}$).

Moreover, factor of putting adsorbent in contaminated studied soil by Arsenic in the study showed significant effect ($p < 0.01$) and positive correlation with contents of As ($r = +87$ to $r = +96$), in the contamination of heavy metals in leaves in 5, 10 and 20 days contact respectively by 2%, 3%, 4% and 5% of Sour lemon peel. The amounts of As adsorbed increased significantly with increase contact time ($p < 0.001$). The other factor of adsorbent percentage and concentration also showed significant agent to decreasing and removal of As from leaves of studied soil samples ($p < 0.001$). The data revealed that even utilizing 1% or 2% of each of bio-adsorbents GP and also SLP can remove more than 90 % and 95.1% respectively of As contents even after 7 days at normal and room temperature. The higher concentration of biomass or time in the rest of study had no significant and meaningful boosting the cleaning-up the contaminated soil samples.

4. Conclusion

The results of current study revealed that biomass from by-products or waste of Food industries can accumulate significantly high levels of Arsenic ($p < 0.0001$) in a short time (3 days) from soil and their uptake rate significantly affected by their concentrations in the contaminated soil ($p < 0.001$). The results revealed that As contents in contaminated soil samples have a bigger decrease at 5% Sour lemon peel (SLP) and 1% or 2% of Grape pomace (GP) concentration. The mixture of SLP and GP can decrease Arsenic content at 99% content in the treated soil after 20 days treatment. The new approach of

biotechnological applications of the citrus by-products is interesting not only from the point of view of low-cost substrate, but also in solving problems related to their disposal. In conclusion, waste of Fruits and crops could be a good adsorbent to remove heavy metals from soil amendments and remediate soils contaminated with toxic heavy metals. Current study provides an optimistic alternative remediation of arsenic contaminated soils emphasizes on low cost and environmental treatment.

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