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Removal of Heavy Metals from *Oryza sativa* Rice by Sour Lemon Peel as Bio-sorbent

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ABSTRACT

Nowadays scientific approach has been diverted towards the biomaterials which are byproducts or the wastes from large scale industrial operations and agricultural waste materials or even vegetable and food processing wastes. The current study was designed for utilization of sour lemon peel as less expensive and much frequently available food waste materials due to prospective metal bio-sorption capacity to toxic heavy metal ions: Nickel, Cadmium and Lead from *Oryza Sativa* rice from Astaneh Ashrafieh, Gillan province in the north of Iran. The effect of soaking rinsed rice samples by NaCl and modified sour lemon peel adsorbent by different concentration, pH, contact time and percentage of adsorbent and association of cooking methods on Nickel, Cadmium and Lead contents were studied. Heavy metal contents in raw, rinsed, soaked by adsorbent and cooked and drained rice samples were determined by Atomic Absorption spectrophotometer. It was found that cooking rice by soaking rinsed rice samples by NaCl 2% and sour lemon peel modified by phosphoric acid 1% at least for 1 hours had the greatest effect (significantly affect $p<0.001$) with regards to lowering Pb and Cd levels in cooked rice. Specifically, it preferentially reduced the Cadmium content by 96.4 %, Nickel content by 67.9% and Lead content by 90.11% from the raw rice, when combined with rinse washing and being soaked by salt for one hour contact time. The results of current study reveal that sour lemon peel as citrus by-products represent a great potential for use as substrates in biotechnological processes.

Key words: Removal Heavy Metal, Sour Lemon peel, *Oryza Sativa* rice, Adsorbent.

INTRODUCTION

Much attention has been made towards adsorbent materials to be used in heavy metal removal from polluted water and soil. Various techniques are applied such as chemical, physical and biological techniques. The technique of plant residues heavy metal ions adsorption was world widely used for waste water treatment[1] such as peat and nut shells, coconut shells, rice husk, tea waste, peanut hulls, almond shells, peach stones, banana peels, and many others[2,3] . Peels of citrus plants contain up to 80% water and the remaining 20% are solid fractions consisting pectin, soluble sugars, cellulose, proteins, phenols. The most abundant represented class of biomolecules in citrus peels are polysaccharides[4] that offer with the presence of pectin a greatest potential for enzymatic or chemical conversion to create desired properties such as ion exchange capacity, galacturoriic acid (GA) the major sugar found in citrus pectin[5]. Over the past few decades, an increasing trend toward efficient utilization of natural resources has been observed around the world. The direct disposal of agro-industrial residues as a waste on the environment represents an important loss of biomass, which could be bio-converted into different metabolites, with a higher commercial value[6]. Many researchers, looking for value-added products, have proposed the use of citrus peels for the production of enzymes[7-14] bioethanol[14-16] , citric acid[14, 17-18], xanthan gum[19-21] single cell protein[22] , prebiotics[23-25] natural antioxidants[26,27], among many others. Approximately 50-60% of citrus fruit is transformed into citrus peel waste[28]. This results in accumulation of large quantities of citrus peel waste as a by-product in citrus-processing industry. Accumulated large quantities of the orange peel waste along with environmental considerations to avoid health hazards derived from unsatisfactory disposal methods addressed the indispensable need for finding alternative biotechnological solutions for waste valorization[29-30]. Sorption field has been enriched by a vast amount of researchers including a variety of citrus biomaterials such as sour orange[31], orange[32-41], lemons[42-45], durian[46], grapefruit[47] and Ponkin mandarin[48].

Heavy metals have been excessively released into the environment due to rapid industrialization and have created a major global concern. Depending on the nature and quantity of the metal ingested, heavy metals can cause serious health problems[49-58]. Their toxicity is related to the formation of complexes with proteins, in which carboxylic acid (–COOH), amine (–NH2), and thiol (–SH) groups are involved. When metals bind to these complexes, important enzyme and protein structures are affected. The most dangerous heavy metals that humans are exposed to are aluminium, arsenic, cadmium, lead and mercury. Aluminium has been associated with Alzheimer's and Parkinson's disease, senility and presenile dementia. Arsenic exposure can cause among other illness or cancer, abdominal pain and skin lesions. Cadmium exposure produces kidney damage and hypertension. Lead is a commutative poison and a possible human carcinogen[59]. Unlike organic wastes, heavy metals are non-biodegradable and they can be accumulated in living tissues, causing various diseases and disorders; therefore they must be removed before discharge. Research interest into the production of cheaper adsorbents to replace costly wastewater treatment methods such as chemical precipitation, ion-exchange, electro-flotation, membrane separation, reverse osmosis, electro-dialysis, solvent extraction, etc.[60] are attracting attention of scientists. Adsorption is one the physicochemical treatment processes found to be effective in removing heavy metals from aqueous solutions.

The emerging process of 'bio-sorption' uses nonviable or viable biological materials to bind contaminants *via* physicochemical mechanisms, whereby factors like pH, size of bio-sorbent, ionic strength and temperature influence metal bio-sorption[61] . Plant wastes are inexpensive as they have no or very low economic value and thereby most of the adsorption studies have been focused on untreated plant wastes. The lemon peel is considered in our study as Lemon is one of the most widely used of citrus fruits not only all around the world but also in Iran. According to the FAO estimates of Iran citrus production for 2005 was 3037.0 thousand tons by 1100 thousand tons of lemon and limes[62] . This juicy fruits has an acidic flavor and it is packed with a number of nutrients and heath benefiting properties. Lemons are also low in calories and contain no cholesterol or saturated fats.

Although Iran is eleventh producer of rice at the world with an annual production 2600000 tons in 2010, during the last years the demand for rice has considerably been increased in comparison with its production, as a result, currently Iran is known as one of the large-scale importer of rice countries[52, 63]. In this study rice was observed for its special individual consumption as a staple food in Iran[52, 64] and probable hazards of its heavy metal contents on population health. Rice variety, treatment of rice and diversity of cooking may affect elemental content and intake of heavy metals[49, 63-64]. The current study deals with the utilization of sour lemon peel as agricultural and food waste materials as bio-sorbents for removal of toxic heavy metal ions: Nickel, Cadmium and Lead from contaminated rice.

MATERIAL AND METHOD

Bio-sorbent

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. 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 20 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 150°C for 24 hours and homogenized in a blender to utilize in adsorption experiments.

Various sour lemon peel (SLP) forms were used, the first was as powder of dried pieces and the second was as carbon active formed from heated SLP in 400 ^{0}c and finally powdered peels that treated by phosphoric acid: 1 % w/v which sieved through 4mm stainless steel sieve. All peel forms were examined for bio-removal of nickel, cadmium and lead from soaking rice samples under various factors such as pH, concentration of SLP and contacting time.

Rice Sampling Method

40 samples of Iranian *Oryza sativa* rice were purchased randomly from 5 popular brands of recognized rice market in 2016 from Astaneh Ashrafieh, in Guilan province near to Caspian Sea, north of Iran (Figure 1).

5 portions of *Oryza sativa* packed in 10 kg portions were mixed before use. All experiments were conducted with 5 replications.

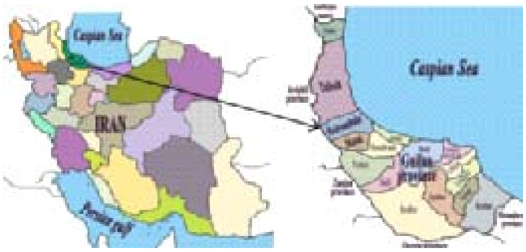

Fig. 1: Region of Agriculture: Astaneh Ashrafiyeh City

Experiment

Nickel, Lead and Cadmium concentrations of Raw, Rinsed , soaked by water / salt, soaked by SLP(sour lemon peel powder) in different contact time/ NaCl, cooked in both sates of treated and untreated by BP association by two different of cooking method were determined by wet digestion method and using 10 g of each rice sample and 25 ml concentrated nitric acid (65% Merck) and 8 ml of Hydrochloric Acid (36.5%, Merck) was added and placed on a hot plate with gradual heat increase to insure full digestion and the disappearance of any residual and nickel, lead and cadmium contents were determined by using flame atomic absorption spectrophotometer (FAAS). Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents by wet digestion method and atomic absorption spectrophotometer analysis based on annual book of ASTM standards and AOAC[65-68].

All digested sample flasks were firstly heated slowly and then vigorously till a white residue is obtained. The residue was dissolved and made up to 10 ml with 0.1 N HNO3 in a volumetric flask.

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. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of Analytical Grade (AR). For heavy metal analyses 5 gram of each rice sample in different states and stages of cooking method was weighed on electronic balance (Shimadzu LIBROR AEX 200G). The samples were analyzed according to standardized international protocols by wet digestion method, Using HNO_3 and HCl , analyzed by a Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals in research Analytical Laboratory in Pharmaceutical Sciences Branch , Islamic Azad University, using six standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines[67-68].

Bio-removal Nickel, Cadmium & Lead from Rice Samples

According to the results of all experiments applied above, current investigation was designed to examine the capacity of lemon peels for the bio-removal of Nickel, cadmium and lead ions from contaminated rice samples after the determination of these metals in such rice samples in different states. In this experiment, 0.100 g powder of dried lemon peels into 3 forms of : as powder of dried pieces and the second was as carbon active formed from heated SLP in 400 ^{0}c and finally powdered peels that treated by phosphoric acid: 1 % v/w , were placed into plastic tank containing 50 g of rinsing rice samples (5 times by distilled water) and 250 ml of deionized water left under laboratory conditions at pH = 6.3 and 25^0 C for almost 1 hour. Half of samples were not treated by SLP in order to find out the potential of bio-absorb.

Bio-sorbed metal concentration (mg/l) and bio-sorption capacity (%) were calculated by using the following equations [49, 64, 69]:

$$\text{Bio-sorbed metal conc. (mg/l)} = C_i - C_f$$

$$\text{Bio-sorption capacity \%} = C_i - C_f / Ci \times 100$$

Where C_i =initial metal concentration and C_f = final metal concentration.

Risk Assessment

To evaluate the potential risk of rice consumption containing the heavy metals, Provisional Tolerable Daily Intake (PTDI) for a 60kg adult person was calculated by the following equation in which C is the heavy metal concentration in rice, Cons is the average consumption of rice in country (110g per capita per day) and BW is body weight of an Iranian adult person (60kg). The output was compared with the WHO/FAO and Iranian standard level.

$$\text{PTDI} = C \times \text{Cons BW}$$

The Iran standard PTDI limits have been recommended for, Cd, Pb and As 0.001, 0.0036 and 0.0021mg/day/kg BW, respectively[70].

Statistical analysis

The values reported here are means of five values. Data were tested at different significant levels using student t-test to measure the variations between the concentration of SLP and contact time parameters before and after treated by lemon peel adsorbent . One way analysis of variance (One-ANOVA) was used for data analysis to measure the variations of heavy metal concentrations using SPSS 22.0 software (SPSS Inc, IBM, Chicago, IL).

RESULTS

Iran Standard (No. 12968) has established the maximum limit of Cd in rice about 0.06mg/kg and on the whole Institute of Standard and Industrial Re-search of Iran set limit of 0.15 mg/ kg as the maximum level for lead and arsenic and 0.06 mg/ kg for cadmium in rice (Organization INS. Food & feed-maximum limit of heavy metals, in 2013.[70]. Although the concentration of Pb and Cd varied among the samples, 60% of the rice samples contained lower limit than the upper level of 0.15 and 0.06 mg/kg recommended by Iran Standard.

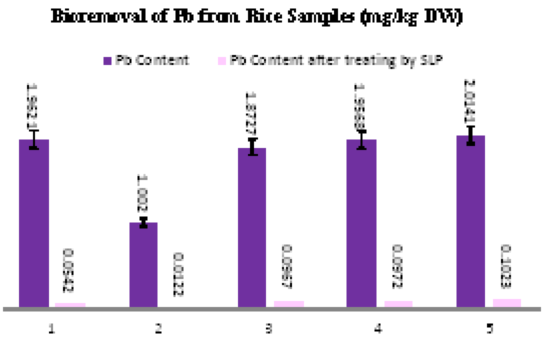

Fig. 2: The mean content of Lead (mg/kg DW ± SD) in cooked rice samples treated by sour lemon peel bio-sorbent in comparison of untreated samples

The range of Cd concentration in studied samples of was 0.017± 0.0005 up to 0.1808 ± 0.032 mg/kg. According to Iran national standards, there was a significance difference between Cd concentration in samples (p=0.008) and according to WHO and FAO standards, there was a significant difference between Cd concentration among Iranian brand rice samples (p=0.001) purchased in different months even in a same brand. According to Iran Standard Organization, lead contamination in Iranian rice was 58.2% , while based on the WHO standard it was 43.2% . Cadmium concentration was higher than Iranian standard level in 35.6 % of Iranian rice samples. Based on WHO standard level

5.9% of Iranian samples were contaminated by Cd. The results of Nickel, Cadmium and Lead contents in 920 samples of raw, rinsing, soaking by NaCl 2%, boiling – drained and cooking rice samples are shown in figures 2 ,3 and 4. All concentrations are expressed as mg /kg DW. ANOVA analysis showed that there was a significant difference in Pb content in rinsing and raw and drained rice samples (p =0.003 and p =0.001 respectively). The maximum Pb content in draining- cooked rice and traditional cooked polished rice belong to brand 5 by 2.0141 in untreated rice by SLP while this sample after soaking by SLP powder and NaCL 2& after 1 hour remarkably being detoxified and Pb content decrease significantly to 0.3542 (mg/kg DW) which shows $p < 0.05$.

The results in figure 3 revealed that all studied boiling - drained and cooked rice samples after treating by bio-absorbent SLP in companion of salt (100%) had lead content less than maximum permissible level 0.15 mg/kg and in all untreated samples except brand 5samples the concentrations of cadmium were over than maximum level which is recommended by FAO/ WHO Expert Committee on Food Additives and national standard[64,70] while treating by SLP resulted in being into safe level.

There were no permitted values available for the other heavy metals such as Nickel to be compared with those of the rice sample contents. The permissible limit set by WHO for Nickel in plants is 10 mg/kg[64]. During the last few decades, a significant increase in environmental contamination with Ni has been observed; hence, a phytotoxic effect of this element, rather than deficiency, is much more commonly found. The increasing Ni pollution of the environment is mainly caused by various anthropogenic activities: fossil fuel combustion, metal (especially Ni) ore mining, smelting and refining, metallurgical and electroplating industry, cement and steel manufacturing, municipal refuse incineration, electrical and electronic industry, chemical and food industry, agricultural use of sewage sludge, application of organic and mineral fertilizers, and many others[71-74]. The maximum permissible Ni concentration in agricultural soil according to the standards set by United Nations Economic Commissions for Europe (UNECE) is 100 mg kg^{-1} and in ground water, 20 µg L^{-1} [75-76]. Ni moves through the environment very easily and is readily taken up by plants. Excessive concentrations of this element are phytotoxic and lead to severe growth inhibition and limited biomass production. The toxic Ni content in plants varies in relation to the degree of sensitivity or tolerance to the metal. It is assumed that a critical toxicity Ni level in sensitive, moderately tolerant, and tolerant species is 10, 50, and 100 mg kg^{-1} dry mass (DM), respectively[69,74,77]. Cereals (especially oat) are recognized as very sensitive to Ni, whereas legumes and members of the mustard family can tolerate and accumulate high amounts of this element. There is little information about Ni toxicity mechanisms in plants, compared to other toxic trace metals like lead (Pb), cadmium (Cd), copper (Cu), and chromium (Cr). This is due to the dual character and complex electronic chemistry of this metal, which makes it

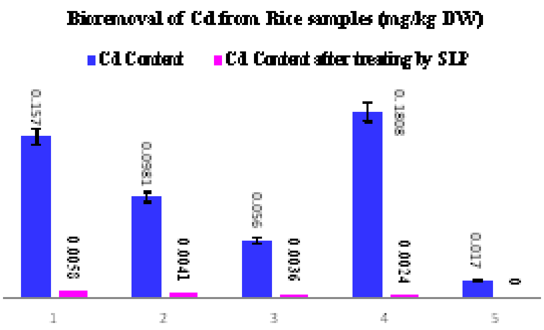

Fig. 3: The mean content of cadmium (mg/kg DW ± SD) in cooked rice samples treated by sour lemon Peel bio-sorbent in comparison of untreated samples

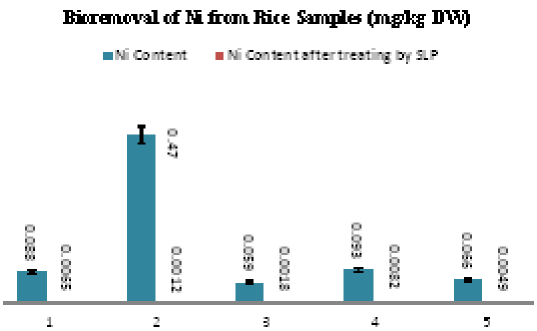

Fig. 4: The mean content of Nickel (mg/kg DW ± SD) in cooked rice samples treated by sour lemon Peel bio-sorbent in comparison of untreated samples

difficult to study its biological role and toxicity [74, 78]. Toxic effects of Ni are observed at multiple levels. One of them is disrupting the nutritional status of plants. Ni interferes with uptake, transport, and distribution of elements of macro- and micronutrients. Literature data show a contradictory effect of Ni on plant mineral nutrition.

The results in figure 4, reveals that treating by SLP reduce nickel content in drained and cooked rice significantly ($p < 0.05$).

DISCUSSION

There was variation in the effectiveness of rinse and washing in removing heavy metals from raw rice . The minimum and maximum Cd contents in rinsing rice and cooked polished *Oryza Sativa* rice were 0.002 and 0.041 (mg/kg DW) in Iranian rice variety respectively . It was found that cooking rice by soaking rinsed rice samples by NaCl 2% and sour lemon peel modified by phosphoric acid 1% at least for 1 hours had the greatest effect (significantly affect $p<0.001$) with regards to lowering Pb and Cd levels in cooked rice. Specifically, it preferentially reduced the Cadmium content by 96.4 %, Nickel content by 67.9% and Lead content by 90.11% from the raw rice, when combined with rinse washing and being soaked by salt for one hour contact time. Rice absorbs heavy metals from the ground and water and this absorption is dependent on the rice type itself and content in the soil rather than on whether the rice is produced organically or not. Agricultural waste materials are usually composed of lignin and cellulose as the main constituents. Other components are hemicellulose, extractives, lipids, proteins, simple sugars, starches, water, hydrocarbons, ash and many more compounds that contain a variety of functional groups present in the binding process. Other studies were also conducted on use of orange and banana peels, rice polish, rice husk and black gram husk in their natural as well as modified form for the removal of cadmium and their relative efficiency was reported. Annadurai et al., in 2002 found out that adsorption of divalent heavy metal ions particularly Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} and Pb^{2+} onto acid (HNO_3) and alkali (NaOH) treated banana and orange peels[79]. The reported maximum adsorption capacities using orange peels were 7.75 (Pb^{2+}), 6.01 (Ni^{2+}), 5.25 (Zn^{2+}), 3.65 (Cu^{2+}) and 1.82 mg/g (Co^{2+}) using orange peel. Acid treated peels showed better adsorption capacities followed by alkali and water treated peels. Based on regeneration studies, it was reported that the peels could be used for two regenerations for removal and recovery of heavy metal ions[79].

Dhakal *et al*. (2005) studied the removal of six heavy metal ions particularly Fe^{3+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Mn^{2+} using orange waste treated with $Ca(OH)_2$ in order to form two types of saponified gels (SOW) (Ca^{2+}-form and H^+-form)[80]. The authors suggested that cation exchange was the main mechanism for the removal of heavy metal ions as the pH of solutions decreased after adsorption. The order of removal for Ca^{2+}-form SOW gel was $Pb^{2+} > Fe^{3+} > Cu^{2+} > Cd^{2+} > Zn^{2+} > Mn^{2+}$. In the case of H^+-form SOW gel, the or-der of removal was $Pb^{2+} > Fe^{3+} > Cu^{2+} > Zn^{2+} > Cd^{2+} > Mn^{2+}$.

In 2000, Ajmal *et al*. (2000) reported that Ni^{2+} had a higher affinity to orange peels than Cu^{2+}, Pb^{2+}, Zn^{2+} and Cr^{2+} and described kinetics of divalent cation adsorption by orange peels with a first-order model with respect to the binding sites. Data for binding of Hg^{2+}, Pb^{2+} and Zn^{2+} by *Citrus sinensis* skin (grapefruit) and coffee husk were in good agreement with the Freundlich isotherm model[14, 81-82]. Schiewer and Patil (2008) studied the removal of Cd^{2+} by fruit wastes (derived from several citrus fruits, apples and grapes). Citrus peels were identified as the most promising bio-sorbent due to high metal uptake in conjunction with physical stability[14, 83]. The metal uptake increased with pH, with uptake capacities ranging between 0.5 and 0.9 meq/g of dry peel[83].

CONCLUSION

Citrus by-products represent a great potential for use as substrates in biotechnological processes. Several studies have been described regarding the employment of this residue for the production of value-added compounds, such as enzymes, biofuels, biopolymers, SCP, organic acids, prebiotic com-pounds and natural antioxidants among others. Biotechnological applications of the citrus by-products are interesting not only from the point of view of low-cost substrate, but also in solving problems related to their disposal. Bio-sorption is a relatively new process that has shown significant contribution for the removal of contaminants from aqueous effluents. In current study the toxic metal ion bio-sorption on inexpensive and efficient bio-sorbent from agricultural and food & vegetable processing waste materials have been investigated as replacement strategy for existing conventional systems. The use of this low cost bio-sorbent is recommended since they are relatively cheap or of no cost, easily available, renewable and show highly affinity for heavy metals. As Literature also reveals that in some cases the modification of the adsorbent increased the removal efficiency, our finding proved that treating lemon peel as a waste material by phosphoric acid 1% could have high potential in removing lead and cadmium from rice as a stable food for most people in Iran and also others around the world. Other waste material from crop and vegetable processing and also agricultural wastes are recommended for future studies as very less work has been carried out in this direction.

Conflict of Interest

The authors have no affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this publication.

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