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Detoxification of heavy metals from *Oryza Sativa* rice by Novel Agro waste: Rice husk treated by coffee bean

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ABSTRACT

Rice is one of the most widely consumed grain crops (besides wheat and maize) in Iran and various countries. Currently, more and more rice is found to be contaminated with heavy metals, which can have a negative impact on human health. The current study aimed to investigate the impact of coffee bean waste (CBW) and Rice Husk (RH) as bio-adsorbent in reducing Lead, Arsenic, Cadmium, and Nickel in rice (*Oryza Sativa*). 950 rice samples were collected from various markets in Tehran to extract the heavy metal content in raw, rinsed, soaked with adsorbent, and cooked and drained rice, determined and measured by wet digestion method. Final concentrations of heavy metals in rice samples were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy according to AOAC International Standards. Batch adsorption experiments were carried out to evaluate the effect of adsorbent dosing, sample state, and ionic strength of solutions on the adsorption of heavy metals onto bio-adsorbents collected simply from legumes and homemade waste at room temperature and then dried at 65 C0 and ground to size mesh 80-100 microns. Arsenic was not found in the samples studied. The cadmium content of the samples was removed through cooking and treated with a mixture of certain percentages of CBW and RH. The content of Lead and Nickel decreased significantly ($p<0.05$) in all cooking methods. The weekly cadmium intake in bulk and unprocessed packaged rice samples is higher than that of Iranian national standards. Still, with the new eco-friendly detoxification method, there is no risk under any cooking conditions and no food contamination. The current research findings indicate that Food/Agro waste can be utilized profitably in processing rice containing heavy metal ions.

Conflict of interest: The authors declare that they have no conflicts of interest.

Introduction

Contamination by toxic heavy metal ions has to turn out to be a major threat as, in large doses leads to acute illness. Because of the growing industrial and agricultural things to do and the fallacious release of metallic ions from wastewaters and home effluents and due to their presence in water and meal resources[1-3], they contain a significant impact on the surroundings and humans' well-being and as a perilous matter can seriously endanger the lives of human and other organisms and cause acute and chronic poisoning. Due to the accumulation of heavy metals, their amounts are not decreased and remain in the soil for thousands of years[4-8]. Heavy metals are not excreted after entering the body but are deposited and accumulated in tissues such as fats, bones, joints, muscles, and eventually cause human diseases such as neurological disorders, cancers, abortion, liver and kidney and brain damage, arthritis, hair loss, respiratory disorders, and Osteoporosis[9-12]. There are elevated levels of heavy metals in soils in many areas of the world, especially in some regions[10]. Heavy metals such as Cadmium (Cd) and Lead (Pb) are the most dangerous toxins around us and enter the natural cycle (soil, water, and air)[13].

The effects of chronic exposure to cadmium toxicity, under protein undernourished state leads to low dietary protein disturbed by cadmium-induced alterations in carbohydrate metabolism and offsets the hepatic and renal process of cadmium detoxification, thereby affecting transfer, storage, or function at the active site of the enzyme or effect a change in the conformation of proteins or nucleic acids required for normal function. Cadmium and zinc appear together in nature, a biologically antagonistic state competing for binding sites on various carrier proteins. Cadmium toxicity and zinc deficiency symptoms affect testicular necrosis, anemia, and weight loss[14-17].

The sorption process is economical, metal selective, regenerative, devoid of toxic sludge generation, metal recovery, and a very effective method. The removal of heavy metals by low-cost adsorbents was encouraging for several natural materials, agricultural and industrial by-products which can be utilized as low-cost adsorbents[18-20].

Biosorption "traditionally" covers the sequestration of heavy metals as well as rare earth elements and radionuclides or metalloids. The "bio" prefix refers to the involvement of living organisms, dead cells, plants, cellular components, or products[21-23]. A lot of metal processing operations and refining industries are considered dangerous sources of heavy metal emissions. Sludge has drawbacks such that it tends to accumulate heavy metals and other persistent toxic compounds coming from industrial discharges, traffic-related pollution, and other commercial activities. Industrial waste has higher heavy metal contents than domestic wastewater. Therefore, toxic metals such as nickel, chromium, lead, cadmium, Arsenic may be found in municipal wastewater/ as well as soil in farmlands due to heavy urbanization and the entry of untreated industrial wastewater into the municipal waste system[24-28]. The importance and applicability of using dead biomass, especially for plants, investigated as a novel biosorbent in comparison with hull husk, on the whole, Agro waste and food waste, especially homemade wastes for heavy metals removal, is a very novel, eco-friendly, and environmentally approach[29-32].

This research builds upon:

- Investigation on the utilization of rice husk treated by Coffee bean waste as agricultural and food waste materials in the role of suitable bio-adsorbent for removal of toxic heavy metal ions,
- Investigation on the Effect of Cooking method and Heating processes in studied rice samples [drained, traditional method in Iran] on heavy metal contents: Lead, Cadmium, and Arsenic.

Method

Rice sampling

Initially, 950 samples of white Hashemi rice (*Oryza Sativa*) were purchased randomly in August 2021 from the Tehran market and prepared in two groups. The first group consisted of 4 samples of Hashemi rice with five well-known brands and was purchased in 1, 5, and 10 kg packages from shopping centers and chain stores in Tehran province. The second group consisted of 6 samples of bulk rice that are sold in kilograms prepared from daily markets in Tehran province. All samples were purchased at the same time. Both groups were combined before the analysis, and the experiments were performed 3 times with repeatability.

Bio-adsorbent Sampling

a) Coffee waste is collected from remarkable local coffee shops in Tehran in June of 2020 and dried up in an oven at 50°C for 48 hours. The studied Coffee bean waste samples were utilized during the Bio-adsorption process in the current investigation for removing heavy metals: Nickel (Ni), Arsenic (As), Lead (Pb), and Cadmium (Cd).

b) Rice husk (RH) was collected from ten major farmland rice production areas in Gilan province in the north of Iran from cities : Rasht, Shaft, Khomam, Masal, Soumahe Sara, Roudbar, Fuman, Astaneh-e Ashrafiyyeh Lashtenesha, Kochesfehan, and each sample consists of 20 subsamples. All sample sites were recorded using a hand-held Global Position System (GPS). The name and descriptions of all the sites in this study are provided in Fig 1.

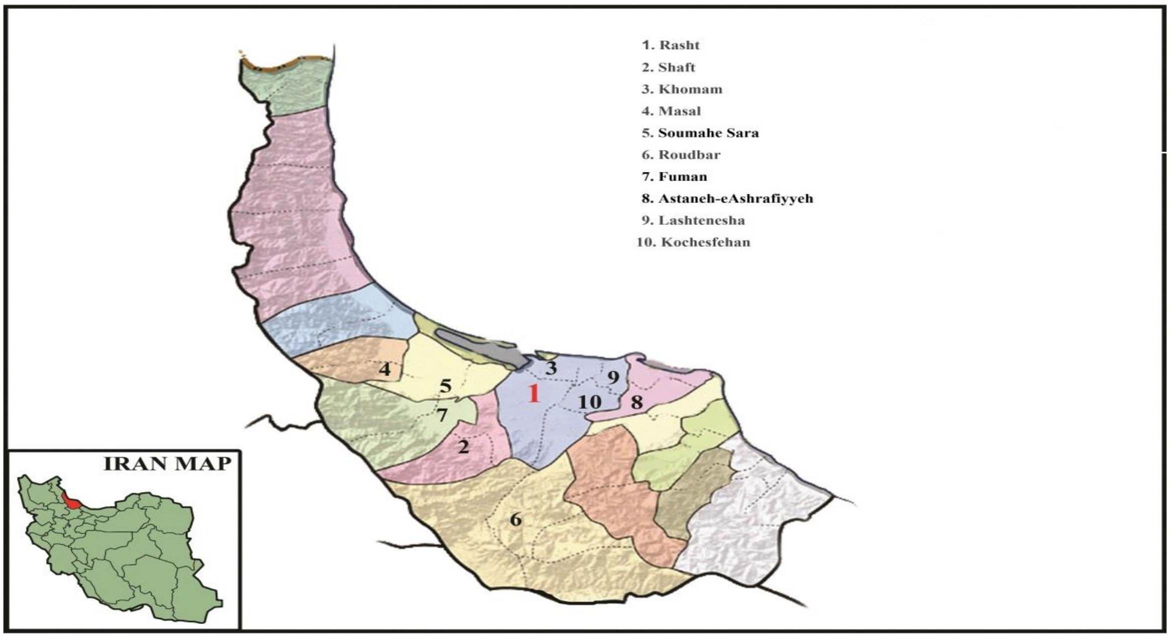

Fig 1. Description of husk rice sampling area

Sample preparation method

In order to prepare the rice samples in the presence and absence of biosoebents (coffee bean waste (CBW) and Rice Husk (RH)), about 50 grams of rice was weighed and placed in the oven at 60°C to remove moisture and determine the dry weight. After drying and reaching to the constant weight, 10 grams of rice was transferred to a 250 ml beaker and kept at 65ºC for 48 hours. Then nitric acid 65% (Merck) and hydrochloric acid 37% (Merck) was added in the ratio of 3:1. After 5 minutes at room temperature, the solution was slowly heated to reach a volume of less than 20 ml and then was volume with nitric acid to 50ml. All these methods have been done in 4 different modes for raw, soaked, cooked, and drained samples in the presence and absence of bio-adsorbent: a specified percentage of coffee bean waste (CBW) and

Rice Husk (RH). Determination of the quantities of Ni, As, Pb, and Cd, in all samples is carried out by the wet digestion method and the inductive coupling plasma mass spectrometry based on international protocols (AOAC) with triplicate replication[33-36].

Risk assessment

For the Iranian community, Rice is the second-high consumed food, while the main consumed food by half of the world's population is rice[37,38]. Provisional Tolerable Daily Intake (PTDI) was conducted for a 60kg adult person to evaluate the potential risk of rice consumption Containing the Cd, As, Pb, and Ni by equation 1[37]. The output was compared with the Iranian standard level and WHO/FAO.

$$PTDI = C \times Cons / BW \qquad (I)$$

C = the heavy metal concentration in rice,
Cons = the average consumption of rice in the country (110g per capita per day),
BW = body weight of an Iranian adult person (60kg)

The Iran standard PTDI limits have been recommended for, Cd, Pb, and As 0.001, 0.0036, and 0.0021mg/day/kg bw, respectively[37].

Statistical analysis

The means of three values are reported as final values. Student t-test at different significant levels was used to measure the variations between the concentration of Heavy metals, before and after treatment by modified rice husk and coffee Bean waste ($p < 0.05$). One-way ANOVA was used to measure the variations of toxic metal concentrations using SPSS 22.0 software (SPSS Inc., IBM, and Chicago, IL).

Results and Discussion

The results of determination of Cadmium and Lead concentrations in the untreated rice as well as rinsing and soaking by 2% Nacl treatment in 3 groups of *Oryza Sativa* (Hashemi) samples without treatment by bio-adsorbent were accomplished by ICP-OES in and reported as a mean concentration in mg/kg in Table 1, all group had 5 subsamples and results are the mean of 5 replicates. All concentrations were expressed as (mg/kg in Dry weight ± SE).

The maximum limit of Cadmium in rice has been established at about 0.06 mg/kg by Iran National Standard (No. 12968) and FAO set 0.2mg/kg, and Iran Standard (No. 12968) has set a Pb intake limit of 0.15mg/kg and WHO/FAO assigned permissible Pb intake limit of 0.3mg/kg respectively[39,40]. The mean contents of Pb and Cd in raw, rinsing, soaking by NaCl 2%, drained, and cooked white Hashemi rice samples are shown in Table 1. It was discovered that rinsing 5 times of rice samples decreases heavy metals significantly ($p \leq 0.05$), although soaking by NaCl 2% removes these metals too, but not significantly ($P \geq 0.05$).

There was variation in the effectiveness of the rinsing process in removing heavy metals from raw rice (Table 1). The minimum and maximum Cd and Pb contents in rinsing rice and cooked polished *Oryza Sativa* rice were shown based on mg/kg DW± SE in Iranian rice variety brands and studied samples, respectively. It was found that soaking rinsed rice samples with NaCl 2% at least for 1 hour had the greatest effect (significantly affect $p<0.001$), it preferentially reduced the Cadmium content from 0.317±0.012 in group 1 by highest contamination up to 0.255±0.015 mg/kg DW± SE. Lead contents in group 3 of samples by much more contamination from the raw rice decreased from 4.576 ±0.108 to2.057± 0.089 mg/kg DW±SE when combined with rinse washing and being soaked by salt for one hour contact time.

Table 1. Cadmium and Nickel concentrations, in studied *Oryza Sativa* rice samples in various cooking methods. Data are the averages of 3 replicates and 10 subsamples of 5 samples.

State	Mean of Pb Content(mg/kg DW ± SE)	Range of Pb content (mg/kg DW ± SE)	Mean of Cd content (mg/kg DW ± SE)	Range of Cd content (mg/kg DW ± SE)
Raw Rice group 1	2.987±0.123	2.109-3.673	0.317±0.012	0.211-0.403
Raw Rice group 2	3.018 ±0.042	2.675-3.056	0.302±0.016	0.267-0.406
Raw Rice group 3	4.576 ±0.108	2.894-4.889	0.314±0.028	0.287-0.416
Rinsed group 1	2.586 ±0.216	2.006-2.783	0.267±0.045	0.237-0.378
Rinsed group 2	2.842 ± 0.102	2.067-2.906	0.245±0.056	0.237-0.365
Rinsed group 3	2.645 ± 0.106	2.016-3.010	0.262±0.058	0.235-0.377
Rinsed Rice group 1, Soak with NaCl 2%	2.001 ± 0.034	1.783-2.087	0.255±0.015	0.202-0.287
Rinsed Rice group 2, Soak with NaCl 2%	2.054 ± 0.256	1.823-2.098	0.248±0.013	0.200-0.266
Rinsed Rice group 3, Soak with NaCl 2%	2.057± 0.089	1.903-2.156	0.251±0.014	0.203-0.261

*SE = Standard Error

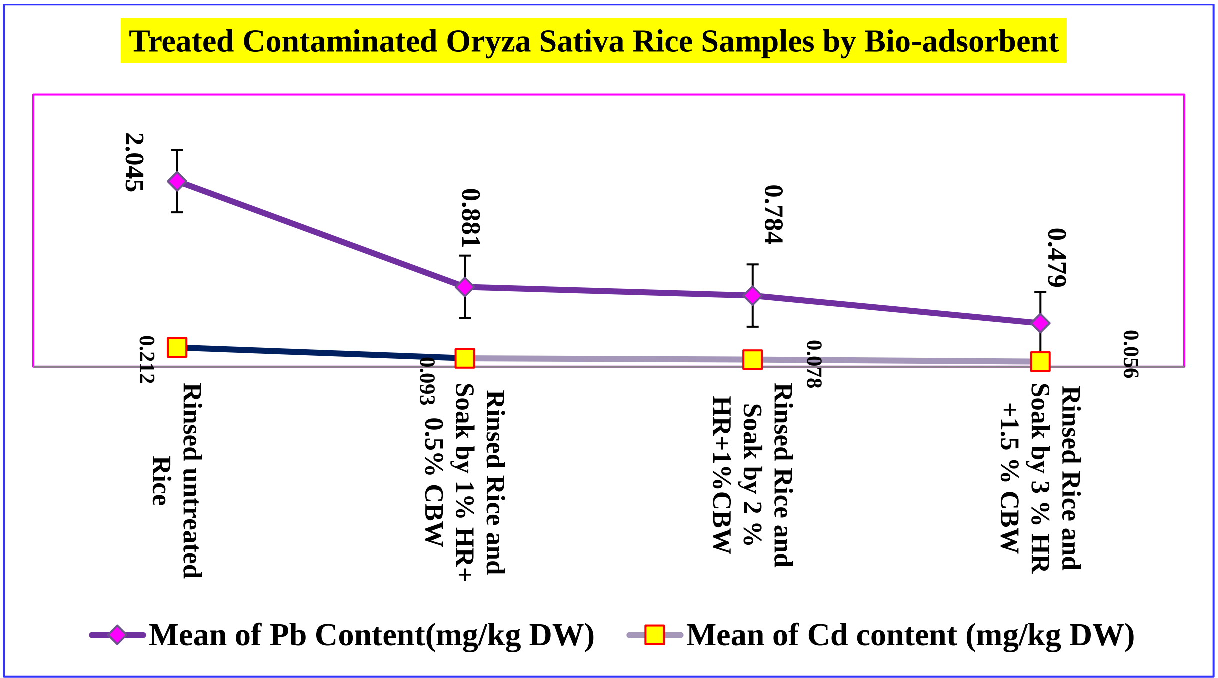

Fig 2. The mean content of cadmium and Lead in Rinsed rice samples 5 times and soaking by NaCl 2% after 1 hour , treated by HR& CBW bio-sorbent in comparison of untreated samples

The heavy metals contents: Lead, and Cadmium of untreated white raw rice and treated samples by RH+ CBW in the Hashemi rice samples were seen in Fig 2, 3, and 4. After pretreatment in the contaminated soil of paddies , it was seen to be detoxified rice samples from Lead in Fig 2. Our data revealed that the mean Pb concentration in studied samples which treated by bio-adsorbents significantly decreased ($p \leq 0.001$). The mean level of lead (mg/kg DW) for all studied rice samples in studied samples without treatment was 2.045 ± 0.005(mg/kg DW), but for treated studied smples by RH+ CBW dependent on the dosage of mass dead biomaterials which soaked by 2% Nacl for 1 hour decreased significantly (P<0.05).

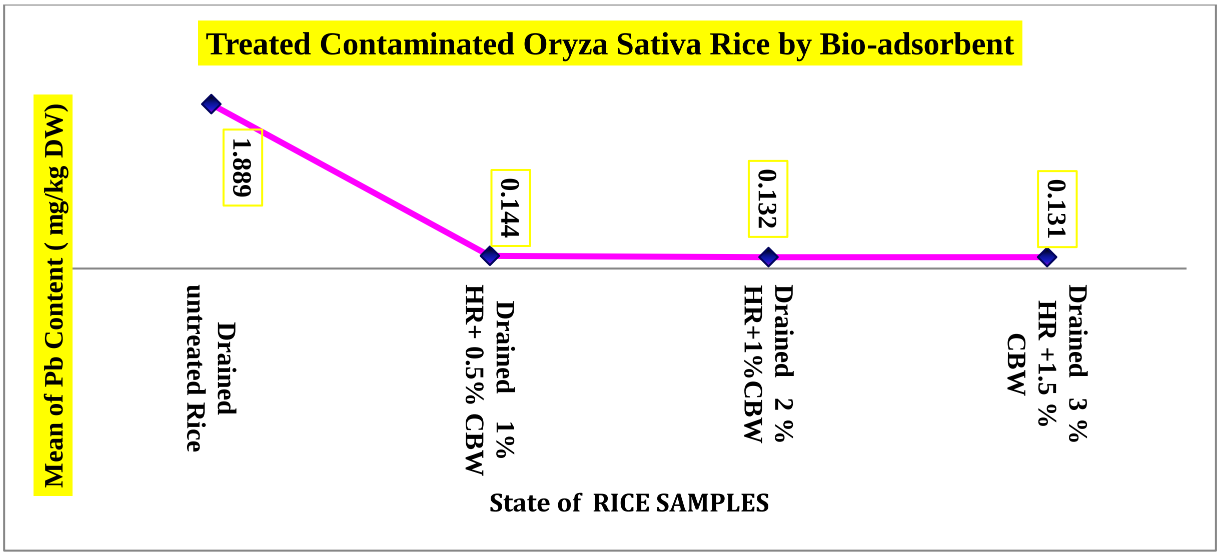

Fig 3. The mean content of Lead in Rinsed rice samples 5 times and soaking by NaCl 2% after 1 hour , then drained 10 minutes which treated by HR& CBW bio-sorbent in comparison of untreated samples

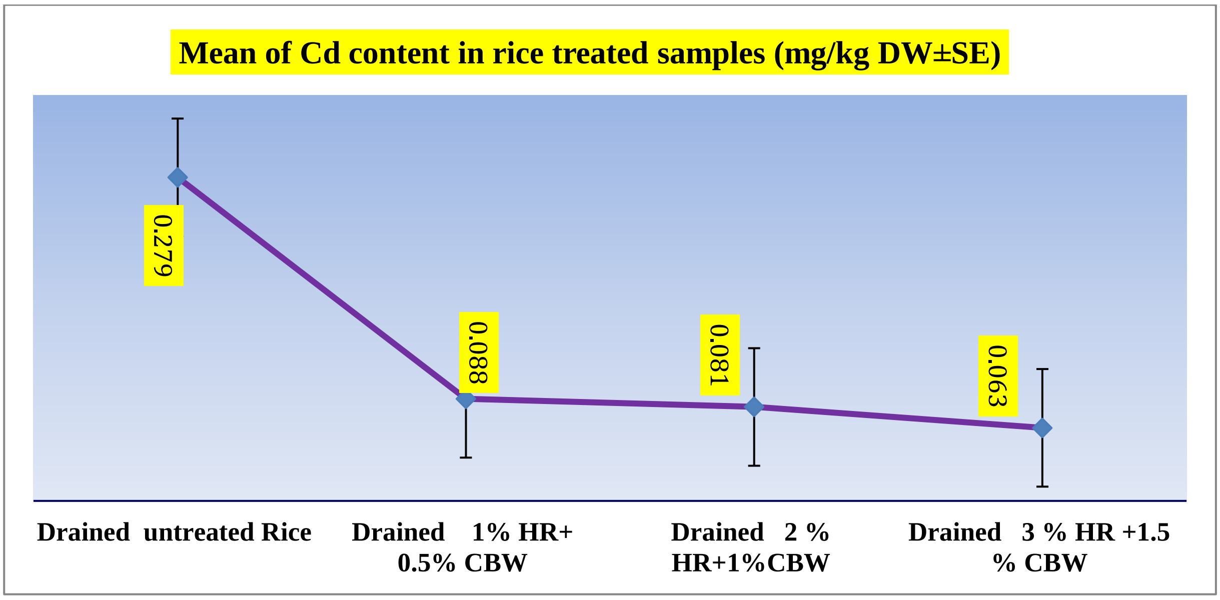

Fig 4. The mean content of Cadmium in 5 times Rinsed rice samples and soaking by NaCl 2% after 1 hour, then drained for 10 minutes which was treated by HR& CBW bio-sorbent in comparison to untreated samples

Phytoavailability of Cd was most dramatically influenced by bio-adsorbent addition (Fig 4 and 5). The mean Cd content in untreated samples 0.279 ±0.021 decreased to 0.088± 0.006 in presence of 1% HR + 0.5% CBW, and 0.081 ± 0.004, then 0.063 ±0.006 by 2% HR+1% CBW and 3% HR + 1.5 % w/w CBW respectively. As it is shown in Fig 5, the mean contents of cadmium in the untreated contaminated white rice samples in comparison to treated samples by 3% HR and 1.5% CBW revealed 100% removal and successful detoxification novel method ($p \leq 0.0001$).

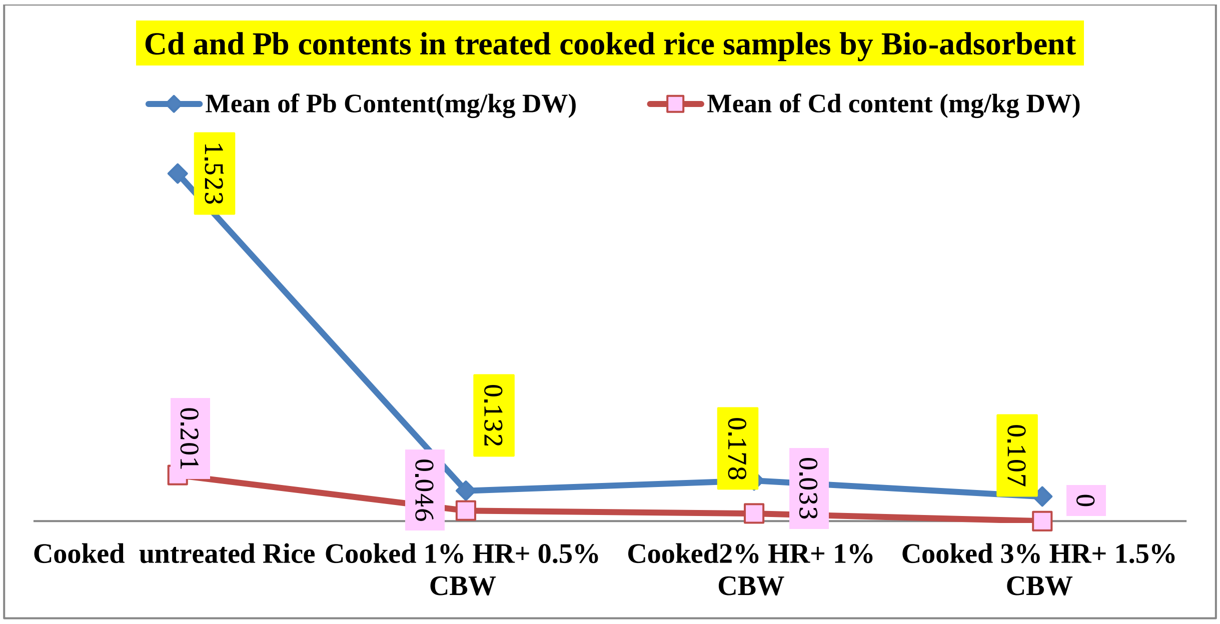

Fig 5. The mean content of Cadmium and Lead in 5 times Rinsed rice samples and soaking with NaCl 2% after 1 hour , then Cooked 10 minutes in the traditional way, which was treated by HR& CBW bio-sorbent in comparison of untreated samples

Results revealed that rice via soaking rinsed rice samples by NaCl 2% and Biosoebents modified, at least for 1 hour, had the best impact with regards to reducing Cadmium and lead stages in cooked rice.

The result of the current study shows more successful detoxification by other studies[1,21,41]. The scientific investigation by Rezafsha et al.[26] diverted towards biomaterials which are byproducts or the wastes from large-scale industrial operations and agricultural waste materials or even vegetable and food processing wastes. The study was designed for the utilization of sour lemon peel as a less expensive and much more frequently available food waste material due to prospective metal bio-sorption capacity to toxic heavy metal ions: Nickel, Cadmium, and Lead from *Oryza Sativa* rice from different farmlands in the northern provinces in Iran. The effect of soaking rinsed rice samples by NaCl and modified sour lemon peel adsorbent by different concentrations, 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 the 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 hour 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 soaked by salt for one hour of contact time. The results of the current study reveal that sour lemon peel as a citrus byproduct represents great potential for use as a substrate in biotechnological processes[26].

The potential of Rice Husk Biosorption in the Reduction of Heavy Metals from *Oryza Sativa* Rice by Soil Detoxification by Lahiji Alidoost et al., in 2016 , focused on the utilization of Husk rice[28].

To manage waste agricultural biomass and convert it into a material resource, the research was carried out in 2016 by Lahiji and the team working. Two field experiments were carried out to evaluate rice (*Oryza Sativa*) productivity in silt loam to which 50 Mg ha-1 of rice husk was added as a control. The field experiment in Guilan province in the north of Iran was conducted to investigate the effect of dry rice husk on the bioavailability of heavy metals: Lead, Nickel, and Cadmium in soil and their accumulation into rice plants and final rice grain product. Phytoavailability of Cd was most dramatically influenced by bio-sorbent addition. Rice husk addition in paddies 10 days before the transplanting rice stems significantly decreased Cd concentrations in rice grain (72.13%). Following bio-sorption addition, lead and Nickel concentrations decreased by 59.53 % and 50.44%, respectively, and increase Potassium, Zinc, and Copper in crop yield significantly ($p< 0.05$), which approve the effect of the uptake of some nutrients in the presence of rice husk. In conclusion, these results highlight the potential for dry rice husks to alleviate the phyto-accumulation of heavy and toxic metal (loid)s and thereby reduce the toxicity and exposure associated with rice consumption.

Conclusion

As metals are nondegradable and highly complex, the wastewater effluents need treatment so as to reduce the heavy metal ions content. The use of commercially available methods has to be replaced by inexpensive and effective low-cost adsorbents. Though metal ions are non-biodegradable, many of them are soluble in aqueous media and easily available for living organisms. The mixture of adsorbents paves way for more efficient re-used through desorption methods for a certain period of time. The use of low-cost adsorbents such as Husk rice, Coffee Bean waste, Banana peels, nut shells, citrus peels, and some other Agro/ Food wastes are potential bio adsorbents for metal remediation, and its usage has increased in the recent past due to its easy availability, low cost, reusability, high efficiency, easy processing, application and recovery without any adverse impact on the environment.

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Author contributions

All authors contributed to the study's conception and design. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.