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Research Article

Novel Detoxification of Lead and Cadmium Contents in (*oryza sativa*) Rice by Pistachio Bio-Waste and Eggshells

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Abstract Due to the accumulation of heavy metal compounds formed in plants for food that lead to human toxicity, and probably high incidence of diseases, ,detoxification of Cadmium and Lead as heavy metals in *Oryza sativa* rice by treated Pistachio Shell (PS) and Eggshells(ES) as two available and novel agro and food bio-wastes has been studied. The originally native rice paddies area was used for years under conventional management for more than 30 years, and the soil was sampled twice (14 and 180 days after incorporating of bio-adsorbents : Pistachio Shell and Eggshell, in 10 hectors in each studied sites). Current assessment was designed principally to appraise the capacity of two bio-adsorbents from food waste sector for the bio-removal of cadmium and lead ions from contaminated soil of paddy rice samples after the determination of these metals in such rice samples in different agricultural sites. Heavy metals in 900 treated and untreated rice samples were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Results were determined as mean ± Standard Deviation of dry weight from three replicates in each test. The mean contents of Cadmium and Lead in most of untreated samples were above maximum level which is recommended by FAO/WHO Expert Committee on Food Additives (JECFA). Lead and Cadmium concentrations in all treated samples by bio-wastes decreased significantly ($p<0.001$) and turned into lower than the maximum permissible limits set by International Committees and also Iranian National Standardization Organization. The findings of present survey indicate that Food /Agro wastes can be utilized in healing of contaminated food stuffs specially rice containing heavy metal ions.

Keywords: Rice, Pistachio Shell, Heavy Metals, Detoxification, Eggshells, bio-waste.

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Introduction

In the hallowed halls of medicine, where the heartbeat of Excessive levels of heavy metals can be harmful to ecosystems due to their ability to accumulate in organisms, cause toxic effects on various organisms (biota), and even lead to mortality in many life forms. Heavy metals such as arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), and thallium (Ti) are metallic elements that have a comparatively high density and are venomous in fewer amounts. Indeed, certain trace elements such as copper (Cu),Selenium (Se),and zinc (Zn) are often classified as heavy metals due to their high density and potential toxicity at elevated concentrations. They are toxic in high concentrations despite being essential for

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maintaining metabolism[1-2].These pollutants can contaminate water sources through various means by the addition of organic compounds, heavy metals, sludge, and sewage organisms; it also includes fungi, algae, bacteria, and hazardous material and chemicals[3-4].Water pollution due to heavy metals has been recognized as a global threat since the industrial revolution with pollutants such as hydrocarbons, heavy metals, inorganic anions, pesticides, nutrient loads, and micro plastic Achieving sustainable and cost-effective water remediation to produce clean water remains a challenging task, particularly in the face of increasing water scarcity and environmental contamination from human activities. Modified agricultural solid waste has been utilized as an effective adsorbent in removing contaminants from wastewater, with chemical treatments enhancing its surface properties [5].The production of activated carbon, bio-char, and charcoal from agricultural residues is emerging as a viable and economical adsorbent option due to their high selectivity, porosity, and surface area [6]. The increase of heavy metals in water and soil both naturally and through human activities will bring problems and since plants are the most important way of transferring heavy metals to the human food chain and biological cycles, the issue of heavy metal contaminations is important [7]. Bio adsorption obtained from agricultural waste and nut shells have the ability to absorb heavy metals from soil and water due to their chemical compounds including pectin. Furthermore, the potential of pistachio shell-based adsorbents for removing metal ions from water solutions is demonstrated under optimal experimental conditions, highlighting their suitability for use in water treatment processes [8-9].

Considerable published researches and surveys on adsorption process using different adsorbents, bio-adsorbents in batch mode have been disclosed from 2 last decades, but these were too few for the process of agricultural innovation and the target of being lengthened. Assumption and utilizing or innovations on enhancing straighten contaminated soils as well as enrichment of nutritive value of cultivated crops, by new approach to agricultural innovations and farming practices is one of the most difficult global issues to combat in the world [10-13].

Applications of the white raw eggshells (bio-waste) as a source of calcium and an environmentally friendly bio-adsorbent on cooking rice samples by the source of calcium phosphates studied by Gorgani and Ziarati in 2020 [14].Their results demonstrated that cooking rice by soaking rinsed rice samples by NaCl 2% and white eggshell significantly reduce Cadmium and Lead consents respectively 90.3% and 91.2% from cooked rice and remedying of contaminated rice samples by this bio-adsorbent suggested as outstanding bio-mass in green and environmentally method. Utilizing eggshells for removing heavy metal ions studied by other scientists too and investigations revealed very magnificent results [15].

Pistachio (*Pistacia vera*) originated from Central Asia. The domestication occurred less than 2000 years ago and traders introduced it throughout the Middle East and the Mediterranean area. Today, the major production areas are located in the Middle East, North America and Europe. Iran is the world's largest pistachio producer and its production accounts for more than 40 percent of the whole world production [16]. There are also some published papers and patents which refer to utilizing nuts shells [17]. Dealing with preceding investigations, pistachio shell was utilized as green and eco-friendly adsorbent for some heavy metals such as Cu (II) removal from aqueous solution in fixed-bed column. Accomplish of different performing parameters such as contrasting influent rate, multiplicity depths of bed and quantifying the concentrations and removal efficiency, different influent concentration on adsorption should be conducted to recognize the most appropriate requirement of the procedure [18].

Total world rice use is seen stagnating at 522.0 million tons in 2023/24, as high domestic and international prices are expected to depress the use of rice for animal feed and deter growth in per capita food intake outside of some Asian countries, Northern America and Europe [19]. USDA Projected at a record-breaking 520.5 million tons (milled basis), global rice production for the 2023/24 period is expected to increase by 12.1 million tons compared to the previous year [20]. The quality control of consumed rice, especially in terms of heavy metals, and the assessment of their impacts on the health of consumers are of particular importance [21-24]. In many studies the accumulation capacity of heavy metals such as Arsenic varies among different types of crops have been demonstrated, as well as in various tissues of the same crop [25].

On the other hand the food and agricultural wastes issues have been considered as one of the most important problems around the world due to the food waste controversy affects every part of the waste management system.The insight of food waste must be analysed clearly as most people may think that the term of food waste is the food we throw away after cooking meals, or the uneaten food from our tables[11,26-28].Food waste, in the situation of an expanding global population and an already suppressed natural environment, is a growing concern, bearing environmental ,economic, and community implications [30]. The magnitude of the problem imminent is investigated by the estimation that 17% of all food produced may be wasted [31-32].On the whole, food waste could be considered as any food-derived perceptible lost during processing in the pattern of residues, faulty batches, retained or analysis samples, or by-products, and that is not recovered for human utilization [11,29].

A number of factors may affect contaminant accumulation such as spices, level and duration of contaminant exposure, topography, agricultural field conditions, amending soil with compost made from municipal sewage sludge and potential bioaccumulation. Due to the accumulation of heavy metal compounds formed in plants for food could be an important factor in human toxicity .

The major source of Cadmium intake for rice eating countries in Asian countries like Iran is rice. It is known that , those who take rice (*oryza sativa*) as staple food for daily energy , exposed to significant amounts of cadmium contents via rice, as rice cropped even from non-polluted areas probably contain Cadmium because of using fertilizers in the farms which have Cadmium[10, 33]. The other major concerns about food waste are pollution by CO_2, in general, farmers and rice processor often burn the food wastes, and causes pollution as releases carbon dioxide (CO_2) into the atmosphere. Research on fully utilize the agriculture/ Food waste means to avoid burning the wastes are worth like. Egg and pistachio shells are very cost-effective raw materials to be used and it is very easy to obtain in Iran. In some countries the main uses of agricultural waste are, animal feed and as fuel in factories. The main consideration of current investigation was detoxification of these heavy metals from *Oryza sativa* rice by treated Pistachio Shell (PS) and Eggshells (ES) as two available and novel agro and food bio-wastes.

Material & Methods

To apprise the environmental pollutants, most notably cadmium and lead in Hashemi (*oryza sativa*) raw rice, 900 Samples(400 treated and 500untreated samples respectively),were gathered from eastern, western and central Guilan at the same time.

Samples were randomly collected from 9 major rice production areas in the north of Iran in 2022-2023 .All samples were collected in during harvesting of rice in Guilan paddy fields in two consequent years. The samples were stored in clean, dry, high density polyethylene bottles of 100 ml capacity with screw caps. 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. Ten gram of powdered sample was weighed precisely on electronic balance (Shimadzu LIBROR AEX 200G) The samples were put in a 100 ml digestion flask and 20 ml of mixture was added to it and heated on a hot plate in the fuming chamber. A digestion mixture comprising of concentrated HNO3 and hydrochloric acid in the ratio of 3:1 was used for wet digestion of the samples [28, 34].Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). This method has been followed in 4 stages for raw rice, rinsing (4 times washing) rice, Boiling and the draining rice, cooked rice [34]. All draining and cooking rice samples have rinsed 4 times then followed by the procedure.

Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents. The 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. Heavy metals in treated and untreated rice samples were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (PerkinElmer, USA), and 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 [35-37].

The current study deals with the utilization of Pistachio Shell as agricultural and Eggshells as food waste materials as bio-sorbents for removal of toxic heavy metal ions: Cadmium and Lead from studied *Oryza Sativa* rice cultivated in 10 self-propelled rice cultivation in Guilan province the north of Iran.

The originally native rice paddies area was used for years under conventional management for more than 30 years , and the soil was sampled twice (14 and 180 days after incorporating of bio-adsorbents: Pistachio shell 1% and Eggshell & 0.5% w/w in 10 hectors). In current survey 18 paddy field areas from Guilan those had different textures or different particle size distribution and bulk density were chosen from 9 main cities. The field experiments were carried out to evaluate rice (*Oryza sativa*) productivity in mostly silt loam to which bio-masses were added as a control and in both groups of survey: treated by RH and conventional rice, the harvesting and all other steps were conducted in the same time and same conditions. The soil samples from all parts of paddy fields with textures of silty clay, silty clay loam, clay loam, and sandy loam were air dried and crushed. Current assessment was designed principally to appraise the capacity of two bio-adsorbents from food waste sector for the bio-removal of cadmium and lead ions from contaminated soil of paddy rice samples after the determination of these metals in such rice samples in different agricultural sites.

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 concentrations of heavy metals with and without treated by bio-adsorbents. 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 and discussion

Results were determined as mean ± SD of dry weight from three replicates in each test. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy. The results of Cadmium and Lead contents in 500 untreated samples of raw , washing , boiling – drained and cooking rice from 9 main agricultural areas in Guilan province; the north of Iran are shown in table 1 and 2 respectively. Results show that the mean content of Cadmium and Lead in all samples from Guilan province is over. These results indicated that the mean value of Lead and Cadmium concentrations in raw rice in 2023, are 1.868 ± 0.044 and 0.380 ±0.012 mg/kg on Dry Wight respectively.

Table 1- Mean content of Lead in untreated rice samples from various agricultural areas from North of Iran in 2023

Agriculture Area	Mean Lead Content (mg/kg dw)±SD **Raw Rice**	Mean Lead Content (mg/kg dw)±SD **Rinsing Rice**	Mean Lead Content (mg/kg dw)±SD **Draining Rice**	Mean Lead Content (mg/kg dw)±SD **Cooked Rice**
Astara	1. 802 ± 0.087	1.481±0.012	1.603±0.024	1.429±0.008
Talesh	1.804±0.063	1.489 ±0.009	1.611±0.017	1.433±0.007
Rezvanshahr	1. 622±0.025	1.378±0.008	1.549±0.008	1.412±0.009
Somesara	1.942 ±0.171	1.592±0.011	1.784±0.024	1.603±0.031
Bandar Anzali	2. 065± 0.072	1.608±0.018	1.702±0.014	1.207±0.006
Rasht	2.328± 0.048	1.912±0.009	1.901±0.009	1.764±0.012
Astane	1.834±0.023	1.497±0.021	1.703±0.007	1.602±0.003
lahijan	1.718±0.032	1.366±0.022	1.561±0.010	1.491±0.007
langrod	1.702±0.028	1.302±0.016	1.667±0.031	1.503±0.005
Total	1.868 ± 0.044	1.844± 0.047	1.477±0.012	1.672 ±0.010
SD= Standard Deviation				

Table 2. Mean Level of Cadmium in rice from various agricultural areas from North of Iran in 2023

Agriculture Area	Mean Cadmium Content (mg/kg dw)±SD Raw Rice	Mean Cadmium Content (mg/kg dw)±SD Rinsing Rice	Mean Cadmium Content (mg/kg dw)±SD Draining Rice	Mean Cadmium Content (mg/kg dw)±SD Cooked Rice
Astara	0.389±0.006	0.361±0.003	0.312±0.003	0. 308±0.005
Talesh	0.391±0.002	0.362±0.004	0.312±0.004	0. 308±0.004
Rezvanshahr	0.361±0.016	0.328±0.007	0.302±0.001	0.259±0.003
Somesara	0.322±0.005	0.296±0.003	0. 327±0.004	0.316±0.004

Bandar Anzali	0.401±0.006	0.341±0.008	0.382±0. 006	0.341±0.005
Rasht	0.428±0.086	0.378±0.002	0.404±0.003	0.388±0.004
Astane	0.376±0.003	0.301±0.002	0. 351±0.002	0. 303±0.002
lahijan	0.378±0.012	0.302±0.002	0.367±0.003	0.313±0.002
langrod	0.378±0.011	0.361±0.001	0.309±0.003	0.300±0.001
Total	0.380 ±0.012	0.337 ±0.005	0.335 ± 0.003	0.315 ±0.003
SD= Standard Deviation				

The results in figures 1 and 2 revealed that all samples (100%) had Cadmium and lead contents above maximum level 0.2 mg/kg and 0.3 mg/kg which is recommended by FAO/WHO Expert Committee on Food Additives (JECFA) .Average concentrations of lead and cadmium in cooking rice is lower than boiling and draining rice and the results show that washing and rinsing rice has the significant role ($p< 0.003$) in decreasing the cadmium and lead contents. Repetitive washing of the rice can greatly reduce level of heavy metal contents.

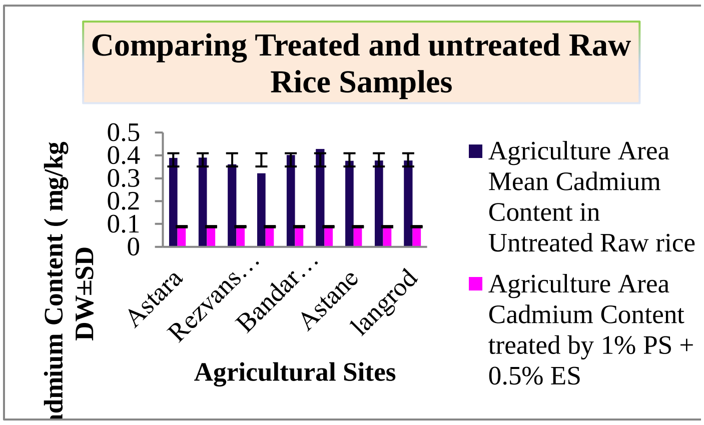

Figure 1- Cadmium content (mg/kg DW) in different forms of (*oryza sativa*) rice samples treated by Pistachio Shell (PS) 1% and Eggshells (ES) 0.5%

Cd contents in all of conventional samples from paddies in Guilan were higher than maximum permissible level set by Iranian National Standardization Organization (ISIRI, 2010)[37],but Cadmium contents in samples from treated soil paddies was lower than maximum permissible level (figure 1). Pistachio hard shells and Egg shells addition 10 days before the transplanting rice stems in paddies significantly decreased Cd concentrations in rice grain (95.8 %), and as it is shown in figure 1, the mean contents of cadmium in all contaminated conventional harvesting white rice samples in comparison of treated samples are much higher and applying PS and ES decreased Cadmium significantly ($p \leq 0.001$). The range of Cadmium contents in treated samples by bio-adsorbents were 0.084 up to 0.092 mg/kg DW , which is much lower than maximum permissible levels.

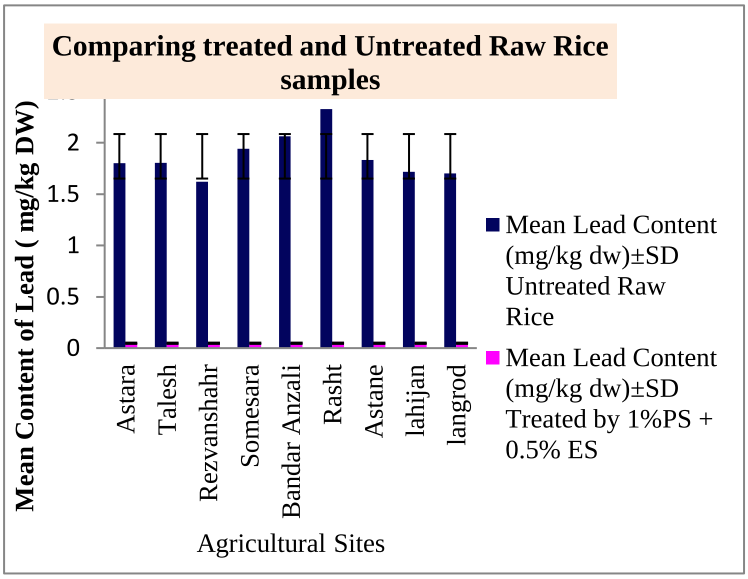

Figure 2- Lead content (mg/kg DW) in different forms of (*oryza sativa*) rice samples treated by Pistachio Shell (PS) 1% and Eggshells (ES) 0.5%

Lead concentration in all of conventional samples from paddies in Guilan were higher than 0.15 mg/kg maximum permissible level set by Iranian National Standardization Organization(ISIRI, 2010) but Lead contents in 97.8% samples from treated soil paddies was lower than maximum permissible level (figure 2). Pistachio hard shells and Egg shells addition 10 days before the transplanting rice stems in paddies significantly decreased Pb concentrations in rice grain and as it is shown in figure 2, the mean contents of Lead in all contaminated conventional harvesting white rice samples in comparison of treated samples are much higher and applying PS and ES decreased Cadmium significantly ($p \leq 0.001$).The range of Lead contents in treated samples by bio-adsorbents were 0.043 in Langarod up to 0.061 mg/kg DW in Rasht .

One of the major environmental pollution in the developing countries like Iran is the heavy metal pollution and the pollution from the use of excessive insecticides, pesticides and fertilizers in the agriculture fields. A questionnaire – based survey on dietary consumption showed that rice was grown in local region is the main source of the foodstuffs consumed by the people in Guilan province. Therefore the problem is rather more serious in Iran and the other developing countries in Asia as most people eat rice as the main foodstuff in their daily diet. By a comparison between acceptable global standards and the level of Cd and Pb on investigated in rice, our results showed that the majority of rice samples had higher level of these heavy metals.

To avoid entrance of metals into the food Chain, municipal or industrial waste should not be drained into rivers and farm lands without prior treatment. A port from treating the discharge that enters into the farms, it is also imperative to utilize alternative measures of cleaning up the already contaminated substrates.

Conclusion

The culmination of this two-year research endeavor addresses the urgent need for effective management strategies against contaminated rice as the most popular staple food. Probably the most important conclusion that could be drawn up from the findings of current study, is that science cereal and rice crops tend to absorb and accumulate Cd in them have been shown to accumulate relatively high concentration of heavy metals in compare to other crops, it is recommended that these type of planets should not be cultivated in the fields which irrigated by urban and industrial waste water or water contaminated by heavy metals. As heavy metal toxicity through contamination of preparation continues to be recognized risk, voluntary programs to provide community education regarding the potential risk of herbal preparations should be supported by the availability free heavy metal testing services. This research provides valuable insights into effective blight management strategies, highlighting utilizing Food Agro bio-waste for healing of contaminated soils.

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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