REMOVAL OF HEAVY METALS FROM AQUEOUS SOLUTIONS BY MEANS OF AGRICULTURAL WASTES: ASSESSMENTS BASED ON BIOLOGICAL ASSAY AND CHEMICAL ANALYSIS

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ABSTRACT

Natural water streams are subject to contamination by various kinds of toxicants including heavy metals which get transferred to water bodies from industrial and agricultural wastes. Elimination of toxic heavy metals from wastewater has often been done using activated carbon (AC) as an adsorbent; however it remains an expensive material. In recent years, different agro-based inexpensive adsorbents have been tested as alternatives to AC. In this context, olive seeds and date palm seeds have been tested here as biosorbents for the removal of cadmium (Cd) and lead (Pb) from artificially contaminated water. The efficiency of metal removal increased as particle size decreased, and vice versa. The bulk sample of olive seeds (grounded materials without sieve fractionation) achieved 95.7% and 97.1% removal for Cd and Pb ions, respectively, compared with 96.1% and 98.1%, respectively for charcoal. The bulk sample of date palm seeds showed similar trend. Our findings based on physico-chemical analyses corroborated with biological evaluation tests in which the decline of metal toxicity (as criteria of removal efficiency) was confirmed by using the *Daphnia magna* assay and the *Vibrio fischeri* (Microtox®) test. Both the agricultural wastes used as biosorbents in this study, are inexpensive and readily available in huge quantities.

Key words: agricultural wastes, heavy metals, wastewater, *Daphnia magna* assay, *Vibrio fischeri* (Microtox®)

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INTRODUCTION

Contamination of aquatic environment by various natural and industrial chemical compounds is being considered as a major problem of global concern (Schwarzenbach et al., 2006). Heavy metals are among the most important contaminants of aquatic ecosystems. They cannot be degraded or destroyed. Human anthropogenic activities are responsible for excessive release of heavy metals into the environment along the food chains, causing detrimental health effects even at minute concentrations. Recently, Mansour (2014) addressed on heavy metals that are of special concern to human health and environment. Their occurrence in the ecosystem arises from rapid industrialization and advances in agrochemical production. These activities have led to wide distribution of heavy metals in the environment, causing health problems to the population due to ingestion of food contaminated by such toxic elements (Zukowska and Biziuk 2008).

Large quantities of metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg) are released into the environment as a result of human activities. Once released, they are distributed among various environmental media and accumulate in food chains, inducing adverse human health effects both at cellular level (Delmas et al. 2000; Fulladosa et al. 2002), as well as at higher levels (Stewart et al. 2003). Metals are the most studied pollutants of soil and water because of their toxicity and long persistence (Liang et al. 2011; Saleem et al. 2014). The major anthropogenic sources of metal contamination include mining, waste disposal, fertilizers, pesticides containing metals such as Cu, Zn and Mn, traffic and domestic emissions, industrial effluents, and atmospheric depositions (Frickel and Elliott 2008).

Domestic and sewage sludge, fertilizers, lubricants, mining and metallurgical activities are among the major sources of cadmium (Cd) release into the environment. Cadmium has long persistence in the environment. The average biological half-life of Cd is about 18 years (Forstner, 1995), and is about 10 years once inside the human body (Salt *et al.*, 1995). Exposure to cadmium poses severe threat to human health. Chronic exposure to cadmium results in kidney dysfunction and exposure to high levels may result in death.

About 80% of lead (Pb) pollution in the atmosphere arises from the use of unleaded petrol. Other sources of Pb include impurities from pesticide and fertilizer formulations, emissions from mining and smelting processes and from combustion of fossil fuels (Sherene, 2010). Lead has a soil retention time of 150–5000 years, and can maintain high concentration for about 150 years after sludge disposal to the soil (Nanda Kumar *et al.*, 1995). Lead can damage the kidney, liver and reproductive system, as well as basic cellular processes and brain functions. Symptoms of lead poisoning include? Symptoms of lead toxicity include anemia, insomnia, headache, dizziness, irritability, weakness of muscles, hallucination and renal damages (Naseem and Tahir, 2001). There are several methods for the removal of heavy metals from contaminated water (e.g., biological, chemical and physical technologies) which are used to reduce the concentrations of these pollutants (Reddy et al., 1999; RAAG, 2000). In parallel to this, different techniques including chemical analyses and biological assessments are used to assess the efficiency of the remediation processes.

In the course of bioassaying efficiency of wastewater remediation, the freshwater cladoceran, *Daphnia magna* Straus arises as one of the oldest and widely used test organisms in aquatic toxicology (e.g., Ortiz et al., 1995; Kaneko, 1996; Seco et al., 2003). These water flea organisms are important link in freshwater trophic chains representing the filter-feeding zooplankton (Mark and Solbe, 1998). *Daphnia magna* as standard test species has several advantageous characteristics, such as their small sizes, easy to culture in the laboratory, short life-span, parthenogenetic reproduction under non-stressed conditions, reproducibility and repeatability of the test results, and relative sensitivity to most chemical compounds (Versteeg et al., 1997; Mark and Solbe, 1998). It's worthy to mention that the *Daphnia* assay has been standardized (OECD, 2004; ISO, 1996), and it is used in routine control of aquatic toxicity assessment of effluents and in environmental safety evaluation of chemical substances (Barata et al., 2006), and in mechanistic studies concerned with aquatic toxicology (Damásio et al., 2008). Therefore, *D. magna* is the most commonly tested freshwater species in acute as well as in chronic tests (Ratte and Hammerswirtz, 2003).

Also, the marine bacteria *Vibrio fischeri*, is one of the most common biosensor used for the risk assessment in aquatic environment based on the inhibition of luminescence produced by the bacteria in the presence of toxic substances. The experimental procedure of toxicity determination using the Microtox® Toxicity Analyzer had been previously described by Kaiser and Ribo (1988) based on the standardized method with *V. fischeri* (ISO, 2009). This bioluminescence based assay is sensitive and rapid, and thus has been long recognized for the regulatory purposes of various inorganic and organic compounds in water samples (Trang et al., 2005; Gueune et al., 2009; Coz et al., 2007).

On the other side, Atomic Absorption Spectrometry (AAS) is an analytical technique that measures the concentrations of elements. It can be used to analyze the concentration of over 62 different metals in a solution and can measure down to parts per billion of a gram ($\mu g/dm^{-3}$) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another higher energy level. AAS is the most widely used technique for heavy metals quantitative analysis in environmental samples (Ortiga, 2002).

The literature presents several biosorbent materials including aquatic and terrestrial plants and microbial sources like algae, fungi, yeast and bacteria. Removal of heavy metals by such biosorbents have many advantages including low cost, high efficiency and sustainability (Paula and Helena 2007; Machado *et al*., 2008).

The aim of this study is to evaluate the efficiency of locally available agricultural wastes as adsorbents for removal of some heavy metals from aqueous solution. This study concerns with seeds of olive and seeds of date palm to test their efficiency in removing cadmium (Cd) and lead (Pb) from water. The tested adsorbents will be compared with charcoal powder, and the efficiency evaluation will base on biological and physicochemical procedures.

MATERIALS AND METHODS

Test organisms: *Daphnia magna* Straus

A single laboratory colony of *Daphnia magna* cultured in our laboratory, at 20 ± 2 °C and 12:12 h light: dark cycle, was used in this study. Bulk cultures of 15 animals each were maintained in ASTM hard synthetic water (Barata et al., 2006) and the animals were fed daily with *Scenedesmus subspicatus* (corresponding to 2 mg C/L; Boersma, 1995). The culture medium was changed every other day and neonates (<24 h) were removed and transferred to 2-L beakers and reared under the same conditions as their mothers until they reached their 4th instars (4-5 days). At this stage, groups of juveniles were collected and used for toxicity studies.

Test organisms: *Vibrio fischeri* bacteria

The Microtox® Acute test exposes a luminescent organism (e.g., *Vibrio fischeri* bacteria) to aqueous samples, and measures the increase or decrease in light output by the test organism. Required reagents contain living luminescent bacteria that have been grown under optimal conditions, harvested, and then lyophilized (freeze-dried). The lyophilized bacteria are rehydrated with reconstitution solution to provide a ready-to-use suspension of organisms. The test system measures the light output of the luminescent bacteria after they have been challenged by a sample and compares it to the light output of a control (reagent blank) that contains no sample. A difference in light output (between the sample and the control) is attributed to the effect of the sample on the organism. The effect of the tested substance is measured in terms of effective concentration (EC) for 50% or any other level of effectiveness. It may also express in terms of inhibition concentration (IC) for 50% or any other level of inhibition.

In the present work, we used the freeze-dried luminescent bacteria, *Vibrio fischeri* (13F4067A), Reconstitution Solution (AFZ686016), Osmotic Adjusting Solution (20%NaCl; AFZ686019), and Diluent Solution (2%NaCl; AFZ686011), which were supplied by Modern Water Inc., New Castle, DE

19720, USA. The tests were performed using the Microtox® Model 500 Toxicity Analyzer from Modern Water Inc. The analyzer was equipped with a 30-well temperature-controlled incubator chamber at 15°C. A small compartment held at 5°C was used to store the bacteria before dilution. The light output was recorded from a digital display.

Tested toxicants

Two heavy metals were used in the present study (e.g., cadmium chloride, $CdCl_2$ and lead chloride, $PbCl_2$).They were purchased as chloride salts of high purity grade from the following sources: $PbCl_2$ (Panreac Quimica SA, Spain), and $CdCl_2 . 2H_2O$ (S.D. Fine-Chem. Ltd. BOISAR, Laboratory Rasayan). Prior to start tests, they were dissolved in deionized water to prepare test solutions, based on metal content in the chloride salt.

Preparation of tested agricultural wastes

About 2 kg portions of each of olive and date palm seeds were collected, washed with distilled water, and kept in wide dishes until dried. The seeds were grounded in Raymond grinder, Model TGM, then half of the grounded material was kept in glass bottles (bulk samples), while the other portions were subjected to sieve analysis (gradation test) using a series of woven wire test sieves, of mesh ranging from >2 mm to <0.05 mm. The sieves were overloaded on a mechanical shaker (JEL 200, Prüfsieb) to assess the particle size distribution. Each separated fraction was weighted and kept in a clean bottle until used. On the other side, charcoal activated powder (PA 121237.1610C M=12.01; 100 mesh particle size powder, pH: neutral) was obtained from Panreac Qumica SA, Barcelona, Spain for comparison purposes.

Olive seeds were fractionated to 6 portions ranging from >2 mm to <0.05 mm, while date palm seeds were fractionated to 4 portions ranging from >2 mm to <0.25 mm. Table 1 shows the percentage of each particle size fraction.

***Daphnia magna* bioassay**

Acute toxicity tests, using the above mentioned heavy metals against *D. magna* were carried out as described below. The heavy metals were dissolved in deionized water to prepare stock solutions which were used to prepare working solutions of different concentrations. Concentrations were expressed in terms of mgL^{-1} (ppm) active ingredients (a.i.). About 60 animals were used for each test divided into five replicates of 10 organisms each plus control for each series of concentrations (5-7). The test animals were placed in glass beakers containing 300 mL of test solution. Mortality (complete immobilization) was counted 30min after exposure, adjusted by Abbott's formula (Abbott, 1925) and subjected to probit analysis (Finney, 1971) to estimate EC50 & 95 values, 95% confidence limits and slopes of regression lines. The latter's were constructed by the aid of an Ld-P Software program. Test methods were performed in general accordance with respective to standardized protocols (OECD, 2000) and

Daphnia were not fed during the assay. The EC_{95} values were used to prepare heavy metal solutions for testing removal efficacy of olive oil and palm date seeds. The toxicity values are shown in Table 2.

Microtox® bioassay

The *Daphnia*-EC_{95} values (Table 2) were used to prepare heavy metal solutions for testing removal efficiency of olive oil and palm date seeds, based on measuring inhibition of *V. fischeri* bacteria, using the Microtox® basic test protocol (Villaescusa et al., 1996) at 15 min exposure period. The method will be described briefly later on.

Column chromatographic elution and assessment

Solutions containing 14.5 and 55.6 mg/L of $CdCl_2$ and $PbCl_2$, respectively (corresponding to EC_{95} values derived from *Daphnia* tests; Table 2), were taken as base for preparation of column chromatographic solutions to test the adsorbent efficiency of the studied raw materials. Glass columns (38cm long x 2.3 cm o.d.), provided with taps were filled to about their mid height using ca. 7 g of adsorbent materials (olive and date palm grounded seeds) which were activated at 100±2 °C in an electric oven before packing. Other columns containing 7 g of activated charcoal (AC) were also prepared. The columns were eluted with 300 ml of the metal solutions. Each eluate was collected in a graduated cylinder and its volume was adjusted to the mark of 300ml by deionized water. The pH of each eluate was measured by using pH pen, then all the collected eluates were subjected directly to *D. magna* and *V. fischeri* tests, as follows:

-To each 300ml eluate, 10 daphnids were added and the % kill was counted after 30min exposure time. This was repeated 3 times.

-For Microtox® assay, 1ml of reconstitution solution and 500 µl of diluent were transferred into each of the cuvette designated as control (reagent blank), as well to those designated as treatment (sample). Ten microlitres (10µl) of the heavy metal eluate was added to the sample cuvettes only. Finally, 10 µl of the reagent (bacterial suspension) was added either to control or to sample cuvettes. A difference in light output (between the sample and the control) is attributed to the effect of the sample on the bacterial organism. By dividing I_o (control reading) to I_t (sample reading), the result was expressed in terms of percent inhibition (% I). The process was repeated 3 times for each tested sample and performed within 15 min exposure time according to the Microtox® basic test protocol (Villaescusa et al., 1996).

Determination of Cd and Pb concentrations in the column eluates.

The obtained eluates after passing through different particle sizes of olive oil and palm date seeds, and AC were subjected to atomic absorption analysis to estimate the concentration of Pb and Cd in each eluent. Also, the actual concentrations of Cd and Pb were determined in solutions not subjected to column elution for comparison purposes. The analyses were performed on Perkin Elmer 2380 Atomic Absorption Spectrophotometer. Samples were prepared according to APHA method (APHA, 1992). Instrument start- up and optimization were carried out as detailed in the operating manual. The source of the flame was a mixture of air-acetylene. The wave length for Pb was 217 nm and 228.8 nm for Cd. Working standard solutions (BDH laboratory reagents, Ltd Poole England) were prepared by appropriate dilution of stock solutions. Each measurement was made in triplicate and the mean of the three values was calculated. Quality assurance was achieved by measuring blank test solutions.

Statistical analysis

The data revealing the efficiency of the particle sizes of olive oil and date palm seeds on toxicity decline or inhibition decline derived from *Daphnia* or Microtox®assays, respectively were subjected to statistical analysis, using GraphPadPrism 5 Demo (www.graphpad.com/downloads/docs/Prism5Regression.pdf), and expressed as means ± S.E. Paired samples (t) test was used to compare the data of the control with those of treatments, where $P < 0.05$ and $P < 0.01$ were considered for significant and high significant differences, respectively. When $P > 0.05$, values were not significantly different from one another.

RESULTS

The toxicity of $CdCl_2$ solution before subjecting it to column chromatographic elution was found 7.1 & 14.5 mgL^{-1} for *D. magna*, in terms of LC50 & LC95 values, respectively. That of $PbCl_2$ solution was 14.87 & 55.6 mgL^{-1}, respectively (Table 2). Solutions containing $CdCl_2$ or $PbCl_2$ at concentrations corresponding to the LC95 (= EC_{95}) values were subjected to column chromatographic elution. The obtained eluates were bioassayed against *D. magna* and *V. fischeri* bacteria according to the above mentioned procedures. Also, concentrations of Cd and Pb were spectrophotometrically determined before and after column chromatographic elution.

Solutions of $CdCl_2$ and $PbCl_2$ possessed pH values of 5.3 and 5.2, respectively (Table 3). This acidic pH tends to neutralize after passing through charcoal or olive seeds, but showed slight alkalinity after passing through date palm seeds (8.3 and 7.9, respectively for Cd and Pb).

Table 4 presents toxicity data in *Daphnia magna* exposed to heavy metal solutions after elution through chromatographic columns packed with different particle sizes of olive seeds, compared with charcoal. In the case of $CdCl_2$, percent kill in *D. magna* accounted to 94.3% for control treatment. Charcoal eluates showed 11.23% kill, but the grounded olive seeds showed kill results mostly higher than that of charcoal value. The data of kill percentages enabled us to estimate the percent decrease of toxicity attributed to the action of the adsorbent materials. Accordingly, the decrease of Cd toxicity due to charcoal accounted to 88.09%; a result which were nearly achieved by the olive seed - bulk sample (88.5%) which represents the grounded seeds without sieve fractionation. The lowest decrease of toxicity (0.2%) was obtained by the fraction of >2 mm-particle size.

Similar trend was obtained for $PbCl_2$ control which achieved 93.1% kill and charcoal eluent which caused 11.97% kill; giving rise to 87.1% decrease in the toxicity of $PbCl_2$ solution due to the metal adsorption on charcoal. The olive seed - bulk sample achieved toxicity decline (83.3%) nearly equal to that of charcoal. Different olive seed-particle sizes caused gradual elevation of the toxicity decrease values with the gradual decrease of particle sizes (Table 4). The pattern of % decrease of toxicity, starting from charcoal and ending at bulk sample, occurred in "U-shape", either for $CdCl_2$ or $PbCl_2$ solutions.

The toxicity data in *Daphnia magna* exposed to heavy metal solutions after elution through chromatographic columns packed with different particle sizes of date palm seeds, compared with charcoal, are presented in Table 5. In the case of $CdCl_2$, percent kill in *D. magna* accounted to 94.3% for control treatment. Charcoal eluates showed 13.1% kill, but the grounded date palm seeds showed kill results mostly higher than that of charcoal value. However, the decrease of Cd toxicity due to charcoal accounted to 86.1%; a result which was nearly achieved by the bulk sample of date palm seed (86.6%). The lowest decrease of toxicity (20.2%) was obtained by the fraction of >2 mm-particle size.

Similar trend was obtained for $PbCl_2$ control which achieved 93.1% kill and charcoal eluate which caused 14.0% kill; giving rise to 84.9% decrease in the toxicity of $PbCl_2$ solution due to the metal adsorption on charcoal. The date palm - bulk sample achieved toxicity decline (84.6%) closely equal to that of charcoal. Gradual decrease of the particle sizes caused gradual toxicity decrease (Table 5). The pattern of % decrease of toxicity, starting from charcoal and ending at bulk sample, occurred in "U- shape", either for $CdCl_2$ or $PbCl_2$ solutions.

Table 6 presents inhibition data in *Vibrio fischeri* bacteria exposed to heavy metal solutions after elution through chromatographic columns packed with different particle sizes of olive seeds, compared with charcoal. The % inhibition decline for $CdCl_2$, with respect to different particle sizes showed values ranging between 88.70% for olive -bulk sample and 92.52% for the >2 mm-particle size. This means that the inhibition decline was generally higher and moves in narrow variations with respect to the different tested particle sizes.

The % inhibition decline in the case of tests with $PbCl_2$ solutions showed typical pattern of that obtained with $CdCl_2$ solutions. In both cases, no significant differences were obtained between charcoal and the bulk sample, with respect to their values of % inhibition decline (Table 6).

Similar trend was obtained for tests with the date palm seeds as adsorbent materials for Cd and Pb in aqueous solutions. The % inhibition decline caused by charcoal was found 86.78% and 87.63% for $CdCl_2$ and $PbCl_2$ solutions, respectively. Those for the bulk samples were accounted to 87.00% and 87.53%, respectively without any significant differences (Table 7).

Concentrations of Cd and Pb was performed by atomic absorption spectroscopy, either before or after elution through chromatographic columns packed with different particle sizes of the tested materials compared with charcoal. Table 8 includes concentrations of Cd in the freshly prepared solution, as well as in the eluates subjected to charcoal and olive seeds of different particle sizes. This enabled us to estimate percent concentration decrease of cadmium, which is indicative to the concentration retained on the adsorbent materials in the column. These values were accounted to 96.1% and 95.7% for charcoal and the bulk sample, respectively without significant difference between them. In between, the metal concentration retained in the column seemed to increase with the decrease of the particle size of the adsorbent material (olive seeds). Similar trend was obtained for Pb; where the concentration retained in the column was 98.1% and 97.1%, respectively for charcoal and the bulk sample without significant difference between both values.

The results of heavy metal estimation in columns packed with charcoal and date palm seeds are presented in Table 9. Percent concentration decrease of Cd and Pb was found 94.7% and 96.2%, respectively in the columns packed with charcoal. The bulk samples of date palm seeds showed very close values (e.g., 94.2% and 94.8, respectively for the two metals).

DISCUSSION

The use of agricultural wastes for wastewater treatment has many benefits including superior and selective adsorption ability of heavy metal ions, and easy regeneration. On the other hand, the accumulation of unused agricultural wastes can cause a number of problems such as elevated chemical oxygen demand (COD) and biological chemical demand (BOD) as well as total organic carbon (TOC) due to discharge of soluble organic compounds contained in the plant materials (Nakajima and Sakaguchi, 1990; Gaballah et al., 1997).The increase of these parameters can cause decline of dissolved oxygen (DO) content in water threatening the aquatic life (Tripathi and Ranjan, 2015).

To better understanding the toxicological profile of environmental toxicants, the impacts of such toxicants should preferably measured by organisms representing different trophic levels (Choi and Meier, 2001). In the majority of aquatic ecosystems, the most important trophic level in terms of energy flow and nutrient cycling is the bacteria. So, the Microtox® assay, based on *Vibrio fischeri* bacteria, has been widely applied as a rapid and economical monitoring tool for toxicity assessment of environmental contaminants (McFeters et al., 1983). On the other hand, acute toxicity testing using daphnids, (e.g., *Daphnia magna*), is a common bioassay used internationally for screening toxicity of chemicals and monitoring of effluents and contaminated waters (Persoone et al., 2009). *D. magna* has been recommended as a standard test organism by many international organizations (e.g., ISO, 1996; OECD, 2004) and has been used routinely in toxicological studies (Biesinger and Christensen, 1972; Hermens et al., 1984; De Schamphelaere et al., 2004). Hence, it is important to include both *V. fischeri* and *D. magna* in a battery of tests designed for protecting the aquatic ecosystems.

In the majority of heave metals remediation research, evaluations were based on analyses conducted by traditional analytical tools such as atomic absorption spectroscopy (AAS) (e.g., Bansal et al., 2009; Murugavelh and Vinothkumar, 2010; Hegazi, 2013; Yusoff et al., 2014). In the present investigation, we combined both biological assays with AAS analyses. As shown from Table 10, Cd and Pb concentrations were highly removed from the eluates due to the adsorption effect of charcoal and olive or date palm seeds. The decrease in metal concentration, as determined by AAS, was accounted to 96.1% and 98.1%, respectively for Cd and Pb by charcoal, and 95.7% and 97.1%, respectively by olive seeds. Nearly similar results were obtained for date palm seeds. Such trend was achieved either by *D. magna* or *V. fischeri* assays. In all cases, the AAS analyses corroborated the biological assays. On the other hand, the bulk samples of olive or date palm seeds (grounded material without sieve fractionation) proved to possess similar removal efficiency as compared with those achieved by charcoal (Table 10). Therefore, the tested agricultural wastes may be considered as adsorbent materials for heavy metal removal from aqueous media. Furthermore, their possible acting as alternatives to charcoal should subject to further investigations.

Jiang et al. (2010) tested the kaolinite clay for removing metal ions from real wastewater containing Pb (II), where its concentration was reduced from 160.00mg/L to 8.00mg/L, giving rise to a concentration decline of 95%; a result which is comparable with our findings regarding to Pb concentration decrease either by charcoal, olive seeds and date palm seeds (Table 10).

Several researchers have studied the use of activated carbon (AC) for removing heavy metals (Jusoh et al., 2007; Kang et al., 2008). Its usefulness derives mainly from its large micropore and mesopore volumes and the resulting high surface area. The high cost of AC limits its use in adsorption. Searching for low-cost and easily available adsorbents to remove heavy metal ions have become a main research focus. Many varieties of low-cost adsorbents have been developed and tested to remove heavy metal ions. Adsorption of low concentration heavy metals by low-cost materials is recognized as an effective and economic method for wastewater treatment, and can act as an alternative to AC (Fu and Wang, 2011).

To date, hundreds of studies on the use of low-cost adsorbents have been published. Agricultural wastes, industrial by-products and wastes and natural substances have been studied as adsorbents for heavy metal wastewater treatment (e.g., Babel and Kurniawan, 2003; Bhattacharyya and Gupta, 2008; Sud et al., 2008; Betancur et al., 2009; Reyes et al., 2009; Sheng et al., 2009).

Biosorption of heavy metals from aqueous solutions is a relatively new process that has been confirmed as a very promising process in the removal of heavy metal contaminants. The major advantages of biosorption are its high effectiveness in reducing the heavy metal ions and the use of inexpensive biosorbents. Typical biosorbents can be derived from non-living materials (e.g., bark, lignin, shrimp, krill, squid, crab shell, etc.), algal and microbial biomass (Apiratikul and Pavasant, 2008). Different forms of inexpensive, non-living plant material such as potato peels (Aman et al., 2008), sawdust (Kaczala et al., 2009), black gram husk (Saeed et al., 2005), egg shell (Jai et al., 2007), seed shells (Amudaa et al., 2009), coffee husks (Oliveira et al., 2008), sugar-beet pectin gels (Mata et al., 2009) and citrus peels (Schiewer and Patil, 2008), etc., have been widely investigated as potential biosorbents for heavy metals.

To the best of our knowledge, no previous studies were carried out on olive and date palm seeds as bio-adsorbents for heavy metals in aquatic media. The total agricultural area of olive trees in Egypt is accounted to 1,100,000 acre which produce about 1.3 million ton, while the total agricultural area of date palm orchards is about 88,000 acre which produce about 1.3 million ton of dates (Ministry of Agricultural and Land Reclamation, Egypt. www.vercon.sci.eg). One could conclude how much quantities of olive and date palm seeds are dumped yearly into the environment as wastes.

The results of the present investigation revealed that the particle size of the adsorbent material has affected the efficiency of heavy metal removal. This was clearly observed in *Daphnia* results (Tables 4 and 5), where the use of fine particles reflected on lower toxicity of the eluate, and vice versa. By other word, the "metal removal" was much pronounced with the finest particles. This may be due to the fine particles provide larger surface area than the coarse particles. Interestingly, the bulk sample (grounded material without sieve fractionation) showed results comparable to that of charcoal. It has been long recognized that the particle size of an adsorbent material affects the removal efficiency of toxic substances (Fu and Wang, 2011). Moreover, biosorption increases the ability of heavy metal accumulation either by metabolically mediated processes or by physico-chemical uptake (Mohanty et al., 2006).

By using rice husk and fly ash as adsorbent materials for removing Pb and Cd ions from wastewater at different concentrations ranging from 20 to 60 mg/L, the maximum

Table 1. The particle-mesh size distribution of the used agricultural wastes as adsorbents

Olive seeds		**Date palm seeds**	
Particle size (mm)	**Percent**	**Particle size (mm)**	**Percent**
> 2	**42**	**> 2**	**43**
2-1	**20**	**2-1**	**21**
1-0.5	**28**	**1-0.5**	**29**
0.5-0.25	**6**	**0.5-0.25**	**7**
0.25-0.10	**3**	**-**	**-**
0.10-0.05	**1**	**-**	**-**

-: no data available.

Table 2. Toxicity of $CdCl_2$ and $PbCl_2$ to *Daphnia magna* after 30 min exposure period.

$CdCl_2$		**$PbCl_2$**	
Concentration ; ppm	% Mortality	Concentration ; ppm	% Mortality
3.0	22.1	6	21.3
6.0	42.8	12	40.7
9.0	70.3	15	55.0
12.0	93.7	20	83.1
LC_{50}	7.1 (6.33-7.5); ppm	LC_{50}	14.87 (13.1-18.9); ppm
LC_{95}	14.5 (11.9-19.1); ppm	LC_{95}	55.60 (45.9-65.1); ppm
Slope	1.5	Slope	2.0

N.B.: Values between brackets are 95% fudicial limits for the estimated LC_{50} and LC_{95}.

Table 3. pH of $CdCl_2$ and $PbCl_2$ solutions before and after elution through column chromatography packed with charcoal, olive seeds and date palm seeds.

Adsorbent	Heavy metal	
	$CdCl_2$	$PbCl_2$
None (control)	**5.3**	**5.2**
Charcoal	**7.5**	**7.5**
Olive seeds	**7.1**	**7.4**
Date Palm seeds	**8.3**	**7.9**

N.B.:

- Either olive or date palm seeds were used as grounded materials without sieve fractionation (Bulk samples).
- Data are means of 3 replicates.

Table 4. Toxicity to *Daphnia magna* exposed to heavy metal solutions after elution through chromatographic columns packed with different particle sizes of olive seeds, compared with charcoal.

Adsorbent materials	$CdCl_2$		$PbCl_2$	
	% kill	% decrease of toxicity	% kill	% decrease of toxicity
Control	94.3±0.73	-	93.1±0.09	-
Charcoal	11.23±0.62	88.09±0.71	11.97±0.03	87.1±0.06
>2 mm	94.1±0.95	0.2±0.9***	91.3±0.71	1.9±0.12***
2-1 mm	91.07±0.55	3.4±0.52***	79.03±0.33	15.1±0.43***
1-0.5 mm	79.2±0.44	16.01±0.11***	62.23±0.41	33.2±0.61***
0.5-0.25 mm	50.8±1.15	46.1±0.73***	41.5±0.27	55.4±0.03***
0.25- 0.1 mm	21.3±0.99	77.4±0.33**	30.63±0.87	67.1±0.1**
0.1-0.05 mm	15.6±0.26	83.5±0.15**	20.93±0.77	77.5±0.05**
Bulk sample	10.8±0.37	88.5±0.84	15.8±0.57	83.3±0.17

N.B.:

- Bulk sample is the grounded seeds without sieve fractionation.

- Heavy metal solutions contained $CdCl_2$ or $PbCl_2$ at concentrations corresponding to LC95 values (refer to Table 2).

- Mortality in daphnids was computed after 30 min of exposure.

- Each value is a mean ± SE of 3 replicates.

- % Decrease of toxicity = (% kill in control - % kill in treatment) x 100

(% kill in control)

- Statistical analysis: Each mean value of % toxicity decrease was compared with the charcoal value (t-test); where * = significant difference at $P \leq 0.05$; ** = high significant difference at $P \leq 0.01$.

Table 5. Toxicity to *Daphnia magna* exposed to heavy metal solutions after elution through chromatographic columns packed with different particle sizes of date palm seeds, compared with charcoal.

Adsorbent materials	$CdCl_2$		$PbCl_2$	
	% kill	% decrease of toxicity	% kill	% decrease of toxicity
Control	94.3±0.73	-	93.1±0.09	-
Charcoal	13.1±0.15	86.1±0.05	14.0±0.64	84.9±0.61
>2 mm	75.23±1.1	20.2±0.1***	66.4±0.87	28.7±0.04***
2-1 mm	65.4±0.74	30.6±0.03***	61.5±0.67	33.9±0.12***
1-0.5 mm	50.4±0.33	46.6±0.06***	41.3±0.34	55.6±0.5***
0.5-0.25 mm	32.57±0.62	65.5±0.5**	31.3±0.72	66.4±0.03***
Bulk sample	12.63±0.41	86.6±0.08	14.3±0.85	84.6±0.70

N.B:

- Bulk sample is the grounded seeds without sieve fractionation.

- Heavy metal solutions contained $CdCl_2$ or $PbCl_2$ at concentrations corresponding to LC95 values (refer to Table 2).

- Mortality in daphnids was computed after 30 min of exposure.

- Each value is a mean ± SE of 3 replicates.

- % Decrease of toxicity = (% kill in control - % kill in treatment) x 100

(% kill in control)

- Statistical analysis: Each mean value of % toxicity decrease was compared with the charcoal value (t-test); where * = significant difference at *P*≤ 0.05; ** = high significant difference at *P*≤ 0.01.

Table 6. Inhibition of *Vibrio fischeri* bacteria exposed to heavy metal solutions after elution through chromatographic columns packed with different particle sizes of olive seeds, compared with charcoal.

Adsorbent materials	$CdCl_2$		$PbCl_2$	
	% Inhibition	% Inhibition decline	% Inhibition	% Inhibition decline
Control	100±0.00	-	100±0.00	-
Charcoal	10.81±0.10	89.19±0.71	12.37±0.03	87.63±0.06
>2 mm	7.48±0.04	92.52±0.9*	10.93±0.71	89.07±0.12*
2-1 mm	7.55±0.03	92.45±0.52*	11.24±0.33	88.76±0.43
1-0.5 mm	7.78±0.04	92.22±0.11**	11.35±0.41	88.65±0.61
0.5-0.25 mm	8.81±0.02	91.19±0.73**	11.55±0.27	88.45±0.03*
0.25- 0.1 mm	9.50±0.03	90.50±0.33*	12.20±0.87	87.80±0.1
0.1-0.05 mm	10.38±0.02	89.62±0.15	12.27±0.77	87.73±0.05
Bulk sample	11.30±0.06	88.70±0.84	12.47±0.57	87.53±0.17

N.B.:

- Bulk sample is the grounded seeds without sieve fractionation.

- Heavy metal solutions contained $CdCl_2$ or $PbCl_2$ at concentrations corresponding to LC95 values (refer to Table 2).

- Inhibition in *V. fischeri* bacteria was computed after 15 min of exposure.

- Each value is a mean ± SE of 3 replicates.

- % Inhibition decline = (% inhibition in control - % inhibition in treatment) x 100

(% inhibition in control)

- Statistical analysis: Each mean value of % toxicity decrease was compared with the charcoal value (t-test); where * = significant difference at *P*≤ 0.05; ** = high significant difference at *P*≤ 0.01.

Table 7. Inhibition of *Vibrio fischeri* bacteria exposed to heavy metal solutions after elution through chromatographic columns packed with different particle sizes of date palm seeds, compared with charcoal.

Adsorbent materials	$CdCl_2$		$PbCl_2$	
	% Inhibition	% Inhibition decline	% Inhibition	% Inhibition decline
Control	100±0.00	-	100±0.00	-
Charcoal	13.22±0.10	86.78±0.71	12.37±0.03	87.63±0.06
>2 mm	6.40±0.33	93.60±0.9**	10.93±0.71	89.07±0.12*
2-1 mm	6.85±0.03	93.15±0.52**	11.24±0.33	88.76±0.43*
1-0.5 mm	7.05±0.03	92.95±0.11**	11.35±0.41	88.65±0.61
0.5-0.25 mm	7.40±0.06	92.60±0.73**	11.55±0.27	88.45±0.03
Bulk sample	13.00±0.00	87.00±0.84	12.47±0.57	87.53±0.17

N.B.:

- Bulk sample is the grounded seeds without sieve fractionation.

- Heavy metal solutions contained $CdCl_2$ or $PbCl_2$ at concentrations corresponding to LC95 values (refer to Table 2).

- Inhibition in *V. fischeri* bacteria was computed after 15 min of exposure.

- Each value is a mean ± SE of 3 replicates.

- % Inhibition decline = (% inhibition in control - % inhibition in treatment) x 100

(% inhibition in control)

- Statistical analysis: Each mean value of % toxicity decrease was compared with the charcoal value (t-test); where * = significant difference at $P \leq 0.05$; ** = high significant difference at $P \leq 0.01$.

Table 8. Concentrations of $CdCl_2$ and $PbCl_2$ before and after elution through chromatographic columns packed with different particle sizes of olive seeds compared with charcoal, based on atomic absorption spectroscopy.

Adsorbent materials	$CdCl_2$		$PbCl_2$	
	Concentration (mg/L)	% Concentration decrease	Concentration (mg/L)	% Concentration decrease
Control	13.1±0.06	-	53.9±0.56	-
Charcoal	0.51±0.006	96.1±0.71	1.04±0.33	98.1±0. 68
>2 mm	7.89±0.006	39.8±0. 62***	40.1±0.78	25.6±0.82***
2-1 mm	5.78±0.005	55.9±0. 65***	33.9±0.91	37.7±1.43***
1-0.5 mm	0.84±0.007	93.6±0.23*	25.7±0.77	52.6±1.61***
0.5-0.25 mm	0.73±0.01	94.4±0.14*	15.51±0.51	71.2±1.53***
0.25- 0.1 mm	0.64±0.006	95.1±0.76	9.03±0.84	83.2±0.76**
0.1-0.05 mm	0.6±0.003	95.4±0.25	5.83±0.93	89.2±0.55**
Bulk sample	0.56±0.02	95.7±1.40	1.57±0.44	97.1±0.77

N.B.:

- Bulk sample is the grounded seeds without sieve fractionation.

- Heavy metal solutions contained $CdCl_2$ or $PbCl_2$ at concentrations corresponding to LC95 values (refer to Table 2).

- Each value is a mean ± SE of 3 replicates.

- % of concentration decrease = Concentration in control - concentration in treatment) x 100

(Concentration in control)

Where, % of concentration decrease is an indicative to the metal retained in the column.

- Statistical analysis: Each mean value of % concentration decrease was compared with the charcoal value (t-test); where * = significant difference at $P \leq 0.05$; ** = high significant difference at $P \leq 0.01$.

Table 9. Concentrations of $CdCl_2$ and $PbCl_2$ before and after elution through chromatographic columns packed with different particle sizes of date palm seeds compared with charcoal, based on atomic absorption spectroscopy.

Adsorbent materials	$CdCl_2$		$PbCl_2$	
	Concentration (mg/L)	% Concentration decrease	Concentration (mg/L)	% Concentration decrease
Control	12.5±0.03	-	52.1±0.09	-
Charcoal	0.66±0.45	94.7±0.24	2.0±0.77	96.2±0.06
>2 mm	7.1±0.12	43.2±1.40***	40.3±0.89*	22.7±0.12***
2-1 mm	6.9±0.33	44.8±1.55***	33.7±0.73*	35.3 ±0.43***
1-0.5 mm	1.0±0.71	92.0±0.61*	20.1±0.66**	61.4±0.61***
0.5-0.25 mm	0.93±0.94	92.6±0.76*	7.9±0.07***	84.8±0.03***
Bulk sample	0.72±0.17	94.2±0.26	2.7±0.45***	94.8±0.17*

N.B.:

- Bulk sample is the grounded seeds without sieve fractionation.

- Heavy metal solutions contained $CdCl_2$ or $PbCl_2$ at concentrations corresponding to LC95 values (refer to Table 2).

- Each value is a mean ± SE of 3 replicates.

- % of concentration decrease = Concentration in control - concentration in treatment) x 100

(Concentration in control)

Where, % of concentration decrease is an indicative to the metal retained in the column.

- Statistical analysis: Each mean value of % concentration decrease was compared with the charcoal value (t-test); where * = significant difference at $P \leq 0.05$; ** = high significant difference at $P \leq 0.01$.

Table 10. Removal efficiency of grounded olive and date palm seeds for heavy metals (Cd & Pb) in aqueous solutions as evaluated by biological and physicochemical assessments and compared with charcoal.

Adsorbent material	As metal concentration decrease (%)[a]				As % toxicity decrease (*D. magna*)[b]				As % inhibition decline (*V. fischeri*)[c]			
	Olive seeds		Date palm seeds		Olive seeds		Date palm seeds		Olive seeds		Date palm seeds	
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
Charcoal	96.1	98.1	94.7	96.2	88.1	87.1	86.1	84.9	89.2	87.6	86.8	87.6
Balk Sample	95.7	97.1	97.2	94.8	88.5	83.3	86.6	84.6	88.7	87.5	87.0	87.5

N.B.:

- Bulk sample is the grounded seeds without sieve fractionation.

[a]Metal concentrations were determined by atomic absorption spectroscopy (data refer to Tables 8 & 9).

[b]Data refer to Tables 4 & 5.

[c]Data refer to Tables 6 & 7.

removal of Pb and Cd ions by rice husk was accounted to 87.18% and 67.92%, respectively, and that by fly ash was accounted to 76.07% and 73.54%, respectively (Hegazi, 2013). Under test conditions of the present investigation, the removal of Pb and Cd ions from water, either by olive seeds or date palm seeds, was found 95% - 97% (Table 10).

It was reported that maximum adsorption of a biosorbent occurs at pH range 3-6 (Özer and Pirinc̦cj (2006). Both Cd and Pb solutions possessed pH of 5.3 and 5.2, respectively. After elution, their pHs shifted from acidic to neutral or slight alkaline based on the type of the adsorbent material (Table 3). The higher pH values (like 7-8) of the eluates may refer to interaction between the carbonate groups present in the adsorbents causing an increase in the negative charge on their surface area which have resulted in attracting the heavy metals (Khan et al., 2001; King et al., 2006).

Adsorbent materials of botanical origin contain cellulose and lignin as cellular components. These constituents have tendency to adsorb heavy metal ions from water (Murugavelh and Vinothkumar, 2010). The mechanism of metals accumulation by such biological materials has been suggested by Paula and Helena (2007). They reported that polysaccharides proteins and lipids in the wall of biomass cell provide functional groups binding the metal ions. The metals uptake by biomass happens during two steps, the stoichiometric interaction is the first step which occurs between the metal ions and the cell components. The other step includes accumulation of the heavy metal on the binding site(s) (Ahlumalia and Goyal 2003).

CONCLUSION

The novelty of the present investigation comes from using two agricultural biosorbents (e.g., olive seeds and date palm seeds) not previously tested, and comparing them with the classic adsorbent, the charcoal. The efficiency of heavy metal removal from aquatic media was evaluated by two aquatic organisms of different trophic levels, as well as spectrophotometric analyses. Fortunately, the two tested biosorbents showed removal efficiency typically equaled to that achieved by charcoal. Both of the used biosorbents, as wastes, are inexpensive and readily available in huge quantities. Therefore, the findings of the present study may encourage further research in this direction.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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