

# Capability of some agricultural wastes for removing some heavy metals from polluted water stocked in combination with Nile tilapia, *Oreochromis niloticus* (L.)

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**Abstract** Heavy metal (HM) pollution is one of the major problems that adversely affect the aquatic ecosystem and inhabiting biota. Heavy metals adsorption by low-cost adsorbents is one of the techniques used for HM removing from polluted water. In the present study, agricultural wastes (AW), i.e., rice straw, sugarcane bagasse, and maize stalks, were washed with distilled water, dried in a dry-oven, cut into small pieces (<0.5 cm long), and immersed at 1.0 g/L in aquaria containing synthetic mixture of lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn). Nile tilapia, *Oreochromis niloticus* (L.), fingerlings (25.2 ± 0.88 g) were stocked at a density of ten fish per 100-L aquarium for 72 h, during which fish were fed on a fish diet containing 25% crude protein ad libitum twice daily. Samples of water, AW, and fish were collected at different times to determine HM concentrations. The HM removal from polluted water was depending on the type of the metal ions, AW, and the contact time. However, HM concentrations in aquaria waters of all AW treatments decreased significantly by increasing contact time up to 24 h after which their concentrations were almost the same. Concentrations of waterborne Pb, Cd, Cu, and Zn in AW-containing aquaria were significantly lower than those of AW-free aquaria. The presence of any AW reduced significantly HM concentrations. In AW-free aquaria, HM-exposed fish accumulated more HM in their body than those reared in AW-containing aquaria. The results of this experiment showed that all AW had the capability to remove HM levels from the polluted water and reduce their bioaccumulation in fish body. However, rice straw was the more efficient adsorbent for all metals.

**Keywords** Nile tilapia · Agricultural wastes · Rice straw · Maize stalks · Sugarcane bagasse · Heavy metals · Bioaccumulation

## Introduction

Heavy metal (HM) pollution is spreading throughout the world due to the expansion of industrial activities and they easily reach the aquatic ecosystem. Unfortunately, HM are non-biodegradable and could deteriorate fish growth and health (Fazio et al. 2014; Savorelli et al. 2016; Abdel-Tawwab 2016; Abdel-Tawwab et al.

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2013, 2016). Thus, scientific methods for HM removal and/or detoxification are essential to improve the health of the fish inhabiting HM-stressed environment. One of these methods is the use of feed supplements that could adsorb HM and/or facilitate their release out of fish body and subsequently could improve fish health (Abdel-Tawwab 2015; Abdel-Tawwab et al. 2015, 2017a, 2017b). Many other treatment technologies such as adsorption have been developed to remove HM from polluted water.

Adsorption is generally known to be one of the most effective techniques for HM removal from wastewater (Benhima et al. 2008; Zafar et al. 2007; Gupta and Babu 2009; Mosa et al. 2011; Chiban et al. 2012; Witek-Krowiak and Reddy 2013). Considerable attention has been paid to the development of effective and low-cost adsorbents. Thus, studies have been done on the use of AW as alternative biosorbents for HM removal from the aqueous effluents (Tarley and Arruda 2004; Mohan and Pittman 2006; Karnitz Júnior et al. 2010; Dos Santos et al. 2011; Chiban et al. 2012).

Nile tilapia, *Oreochromis niloticus* (L.), is one of the most known freshwater fish species in Egypt and worldwide due to its good growth and high-value market. This fish inhabits different aquatic ecosystems, which may be HM-polluted. On the other hand, some countries use wastewater in fish farming and polluted water may enter the fish farms (Easa et al. 1995; Shereif and Mancy 1995; Shereif et al. 1995). The HM pollution definitely deteriorates fish health and subsequently human health if the fish are consumed. Hence, a low-cost method for HM removal is essential to improve the health of aquatic ecosystem and subsequently the health of the farmed fish. Therefore, the present study was conducted to investigate the capability of rice straw, sugarcane bagasse, and maize stalks to remove HM from the polluted water and reduce their bioaccumulation in Nile tilapia.

## Materials and methods

### Preparation of the adsorbents

Rice straw, sugarcane bagasse, and maize stalks were collected from a local area (Abbassa, Abo-Hammad, Sharqia, Egypt). They were washed with distilled water many times, dried in a dry-oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA) at 85 °C until constant weight, and cut into small pieces (<0.5 cm long). Afterward, these wastes were used without any pre-treatment as adsorbents for HM.

### Preparation of synthetic mixture of heavy metals

Several wastewater samples were collected randomly from Saft-El-Henna drainage (10 km North-East Zagazig city, Sharqia, Egypt), stored in polyethylene bottles, decanted, and filtered on Whatman paper (0.45 mm porosity). Concentrations of lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn) were determined as described. Synthetic mixtures of HM, containing the same concentrations as in the drainage samples, were prepared using lead nitrate ( $\text{Pb}(\text{NO}_3)_2$ ), cadmium chloride ( $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ), zinc sulfate ( $\text{ZnSO}_4$ ), copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; Merck & Co Inc., Kenilworth, NJ, USA).

### Fish rearing and adsorption study

This study was based on a  $4 \times 6$  factorial design with four adsorbents (control, rice straw, sugarcane bagasse, and maize stalks) and six contact times (0, 3, 6, 24, 48, and 72 h) in triplicates. Nile tilapia, *O. niloticus* (L.), fingerlings were obtained from the nursery ponds of the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were transferred to the wet laboratory and kept in an indoor fiberglass tank for 2 weeks for adaptation to the laboratory conditions where light–dark photoperiod cycle was maintained at 12–12 h using fluorescent tubes as a light source. During the adaptation period and the experimental running, fish were fed on a fish diet containing 25% crude protein as used by Abdel-Tawwab (2016) ad libitum twice daily at 9:00 and 14:00 h. The synthetic HM mixture was added to each 100-L aquarium and vigorously stirred. Then, fish ( $25.2 \pm 0.88$  g) were distributed into aquaria at a density of 10 fish per aquarium. Each aquarium was supplied with compressed air via air-stones using aquarium's air pump.



Each AW was kept in a net (<0.5-mm pore) and inserted in each corresponding aquarium at 1.0 g/L. At each contact time, samples from aquarium's water, AW, and whole-fish body were taken for HM determination.

### Water quality parameters

Water samples were collected at each contact time from each aquarium to monitor different water quality parameters. Water temperature and dissolved oxygen were measured in site using a portable oxygen meter (Jenway, London, UK). The pH was measured using a pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). The unionized ammonia (NH<sub>3</sub>) was measured using Multi-parameters Ion Analyzer (HANNA Instruments, Rhode Island, USA). Total alkalinity and total hardness were determined by titration according to Boyd (1984).

### Heavy metals residue

For measuring HM concentrations in water, 1-L water sample was filtered via 0.8 µm Millipore acetylcellulose filter paper (Millipore, Bedford, MA, USA), digested with 10 ml concentrated H<sub>2</sub>SO<sub>4</sub> on a hot plate at 70 °C, concentrated to 50 ml, and transferred to a volumetric flask. Samples were adjusted up to 100 ml with redistilled water.

For measuring HM concentrations in AW and whole-fish body, a 1.0-g sample was oven-dried at 85 °C until constant weight and ashed in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 h. Ash was digested with 5 ml concentrated H<sub>2</sub>SO<sub>4</sub> and gradually kept at 130 °C on a hot plate until complete dryness. Then, the digests were diluted with 2 N HCl to a constant volume. Heavy metal concentrations were determined by using an atomic absorption spectrophotometer (Thermo 6600, Thermo Electron Corporation, Cambridge, UK), which was calibrated using standard solutions.

### Statistical analysis

The obtained data were subjected to two-way ANOVA to evaluate effects of agricultural wastes and contact time as factors. Differences between means were tested at the 5% probability level using Duncan test. All the statistical analyses were performed using SPSS program version 20 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

## Results and discussion

Water quality parameters (Table 1) did not show significant differences among the different treatments ( $P > 0.05$ ) and they were within the acceptable ranges for fish culture (Boyd 1984). The metal concentrations used in the present study were 58.4 µg/L, 1.31 mg/L, 22.15 µg/L, and 2.72 mg/L for Pb, Cd, Cu, and Zn, respectively (Table 2). Only, Cd concentration exceeded the permissible limits (USEPA 1986; WHO 1989).

Metal concentrations in aquaria's water were significantly affected by AW, contact time, and their interaction ( $P < 0.05$ ; Table 2). In addition, concentrations of waterborne Pb, Cd, Cu, and Zn in AW-containing

**Table 1** Changes in physico-chemical parameters of aquaria's water stocked with different agricultural wastes in combination with Nile tilapia exposed to synthetic heavy metals mixture for different contact times

	3 h	6 h	24 h	48 h	72 h	<i>P</i> value
Water temperature (°C)	26.4 ± 0.22	26.8 ± 0.09	26.6 ± 0.06	26.7 ± 0.22	26.6 ± 0.13	0.192
Dissolved oxygen (mg/L)	5.8 ± 0.11	5.9 ± 0.12	5.7 ± 0.12	5.9 ± 0.09	5.8 ± 0.14	0.530
pH	7.65 ± 0.022	7.68 ± 0.031	7.68 ± 0.054	7.68 ± 0.031	7.67 ± 0.021	0.081
Unionized ammonia (mg/L)	0.55 ± 0.022	0.54 ± 0.016	0.56 ± 0.025	0.53 ± 0.025	0.59 ± 0.015	0.074
Total alkalinity (mg/L)	158.3 ± 1.67	160.0 ± 2.58	160.0 ± 2.01	160.0 ± 2.88	155.8 ± 1.54	0.371
Total hardness (mg/L)	244.2 ± 4.91	244.2 ± 4.91	253.3 ± 3.08	250.0 ± 4.83	249.2 ± 2.71	0.482



**Table 2** Concentrations of lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn) in aquaria's water stocked with different agricultural wastes in combination with Nile tilapia exposed to synthetic heavy metals mixture for different contact times

Exposure time (h)	Wastes	Lead ( $\mu\text{g/L}$ )	Cadmium ( $\text{mg/L}$ )	Copper ( $\mu\text{g/L}$ )	Zinc ( $\text{mg/L}$ )
Control					
0		56.5a	1.12a	21.45a	2.22a
3		38.9b	0.59c	15.66b	0.70cd
6		33.7cd	0.51cd	12.18cd	0.61de
24		29.6e	0.45def	12.00de	0.51efgh
48		27.3ef	0.41defg	10.95de	0.49efgh
72		24.2f	0.39efgh	10.58de	0.47fghi
Rice straw					
0		56.5a	1.12a	21.45a	2.22a
3		37.1bc	0.51cd	10.16def	1.06b
6		27.0ef	0.43def	9.11efgh	0.77c
24		25.1f	0.39efgh	7.19ghi	0.50efgh
48		24.6f	0.38efgh	7.17ghi	0.43ghi
72		24.1f	0.34gh	6.50hi	0.40hij
Sugarcane bagasse					
0		56.5a	1.12a	21.45a	2.22a
3		30.2de	0.58c	10.58de	0.60def
6		17.7g	0.58c	6.54hi	0.55efg
24		15.2gh	0.50cd	6.51hi	0.52efgh
48		15.1gh	0.48cde	5.92i	0.51efgh
72		15.1gh	0.48cde	5.48i	0.50efgh
Maize stalks					
0		56.5a	1.12a	21.45a	2.22a
3		26.2ef	0.69b	14.78bc	0.78c
6		16.6g	0.58c	9.62defg	0.36ijk
24		14.2gh	0.45def	7.38fghi	0.28jkl
48		12.1h	0.35fgh	6.32hi	0.26kl
72		11.9h	0.29h	4.74i	0.23l
Pooled SE		1.769	0.031	0.668	0.079
Two-way ANOVA		<i>P</i> value			
Agricultural wastes (AW)		0.0001	0.0001	0.0001	0.0001
Contact time		0.0001	0.0001	0.0001	0.0001
AW $\times$ contact time		0.0001	0.008	0.013	0.0001

Means followed by the same letter are not significantly different

aquaria were significantly lower than those of AW-free aquaria. Moreover, metal concentrations in AW were significantly affected by AW, contact time, and their interaction ( $P < 0.05$ ; Table 3). Concentrations of all tested metals in AW increased significantly by time up to 24–48 h after that their concentrations were approximately the same (Table 3). These results could be attributed to the availability of adsorbing site (surface functional groups) on the adsorbent surface. Additionally, AW may be fermented within days and subsequently their adsorption capacity was low. Similar results with rice straw were obtained by Swelam et al. (2016) who found that the Cu adsorption by straw rice increased significantly with increasing contact time and became almost constant for  $\sim 3.5$  h.

All AW adsorbed metals, but rice straw, were the most efficient adsorbent for all metals. It is clear that metal removal was depending on the type of the metal ions, the adsorbent, and the contact time. Each AW contains many compounds, which have a variety of functional groups present in the binding process, for example carboxyl, amino, alcohol, and esters (Gupta and Ali 2000). These functional groups have the ability



**Table 3** Concentrations of lead, cadmium, copper, and zinc (mg/g dry weight) in different agricultural wastes stocked in combination with Nile tilapia exposed to synthetic heavy metals mixture for different contact times

Exposure time (h)	Wastes	Lead	Cadmium	Copper	Zinc
	Rice straw				
0		0.23j	0.65g	0.28f	1.32h
3		1.50e	2.89f	0.74e	2.71g
6		2.41c	6.91e	1.28d	5.36f
24		3.04b	14.72d	1.64b	10.39c
48		3.48a	35.49a	1.98a	14.85a
72		3.64a	35.68a	2.07a	15.31a
	Sugarcane bagasse				
0		0.27j	0.72g	0.26f	1.37h
3		0.87g	2.11f	0.61e	2.59g
6		0.94fg	6.73e	1.15d	5.45f
24		1.94d	13.23	1.43c	6.14ef
48		2.48c	21.66c	1.48c	7.02de
72		2.52c	23.99c	1.74b	8.10d
	Maize stalks				
0		0.15j	0.37g	0.22f	1.28h
3		0.42i	2.30f	0.68e	2.98g
6		0.68h	6.07e	1.19d	5.28f
24		0.82g	13.66d	1.48c	11.14c
48		0.93fg	26.22b	1.74b	13.78bc
72		0.97fg	29.61b	1.96a	14.18ab
	Pooled SE	0.162	0.225	0.191	0.960
	Two-way ANOVA	<i>P</i> value			
	Agricultural wastes (AW)	0.0001	0.0001	0.0001	0.0001
	Contact time	0.0001	0.0001	0.0001	0.0001
	AW × contact time	0.0001	0.001	0.0001	0.0001

Means followed by the same letter are not significantly different

to bind metal ions in solution or by donation of an electron pair from these groups to form metal complexes. Due to the differences in chemical compositions and functional groups of each AW used in the present study, the adsorption capability differs. Many researchers found a relationship between the presence of various functional groups and their complexation with metals during biosorption process (Tarley and Arruda 2004). The AW capacity for metal removal depends on many factors such as metallic elements, pretreatments of AW, and operating conditions; however, AW tended to prefer some metals more than others (Nguyen et al. 2013). Benaissa (2006) investigated the capacity of four inexpensive materials which are peels of peas, broad bean, medlar and fig leaves, to remove Cd from aqueous solutions; it was noted that the broad bean peel has the maximum adsorption capacity for Cd.

In the present study, rice straw was more effective adsorbent than sugarcane bagasse and maize stalks. The higher adsorption capacity of rice straw is probably due to the presence of silanol (SiOH) groups in its structure and more surface area (Fatemeh et al. 2008). In this regard, Osman et al. (2010) reported that rice hull showed highest efficiency in sequestering Zn, Cd, and iron among biosorbents investigated and the removal efficiencies by rice hull, sawdust, sugarcane bagasse, and wheat straw were 98.2, 96.9, 93.0, and 91.2%, respectively. Additionally, the difference in AW capacity to adsorb metals could be attributed to the abundant availability of binding sites, which enhances the retention of metals onto AW surface (Marin-Rangel et al. 2012; Jiménez-Cedillo et al. 2013; Nguyen et al. 2013). Kelly-Vargas et al. (2012) reported that lemon peel and orange peel demonstrated adsorption capacities for Cu and Pb were 48 and 15% higher than banana peel, respectively, and the Cd uptake by banana peel was higher than that of lemon peel and orange peel 82 and 57%, respectively. On the other hand, Mosa et al. (2011) reported that the removal efficiency of metals



decreased in the order of cotton stalks, maize stalks, and rice straw. They attributed highest removal by cotton stalks to its highest concentration of cellulose, hemicellulose, and lignin as compared to other crop-residues.

All AW adsorbents reduced significantly HM concentrations in whole-fish body ( $P < 0.05$ ; Table 4). In AW-free aquaria, HM-exposed fish accumulated more HM than those reared in AW-containing aquaria. These results suggest that AW immersion in aquaria stocked with Nile tilapia could reduce metal bioaccumulation in their body. It also noticed that metal concentrations in fish body were higher than those in polluted water. Concentration of Pb, Cd, Cu, and Zn in whole-body of Nile tilapia 72-h post exposure in AW-free aquaria were 0.714, 5.47, 1.309, and 7.37 mg/g dry weight, respectively, (Table 4). However, Pb concentrations (0.267, 0.286, and 0.290 mg/g dry weight), Cd concentrations (3.98, 3.52, and 3.58 mg/g dry weight), Cu concentrations (0.284, 0.262, and 0.283 mg/g dry weight), and Zn concentration (3.27, 3.64, and 3.13 mg/g dry weight) in fish body were approximately the same when fish were stocked with rice straw, sugarcane bagasse, and maize stalks for 72 h, respectively.

**Table 4** Concentrations of lead, cadmium, copper, and zinc (mg/g dry weight) in whole-body of Nile tilapia exposed to synthetic heavy metals mixture in combination with different agricultural wastes for different contact times

Exposure time (h)	Wastes	Lead	Cadmium	Copper	Zinc
	Control				
0.0		0.031g	1.67e	0.082h	1.42f
3		0.372d	3.41c	0.412d	4.23c
6		0.449c	4.23b	0.617c	5.62b
24		0.575b	4.84ab	0.975b	6.39ab
48		0.656ab	5.22a	1.249a	6.91a
72		0.714a	5.47a	1.309a	7.37a
	Rice straw				
0.0		0.031g	1.67e	0.082h	1.42f
3		0.112f	1.98de	0.102g	1.83ef
6		0.175ef	2.23d	0.161fg	2.28e
24		0.218ef	2.84d	0.216ef	2.73de
48		0.251ef	3.52c	0.252ef	3.13d
72		0.267ef	3.98c	0.284e	3.27d
	Sugarcane bagasse				
0.0		0.031g	1.67e	0.082h	1.42f
3		0.135f	1.72e	0.125g	1.83ef
6		0.190ef	1.83de	0.174fg	2.24e
24		0.231ef	1.97de	0.213ef	2.83de
48		0.263ef	2.38de	0.243ef	3.18d
72		0.286e	2.86d	0.262ef	3.64d
	Maize stalks				
0.0		0.031g	1.67e	0.082h	1.42f
3		0.113f	1.81de	0.132g	1.78ef
6		0.175ef	2.31d	0.198fg	2.10e
24		0.228ef	2.92d	0.237ef	2.97de
48		0.262ef	3.46c	0.261ef	3.05d
72		0.290e	3.58c	0.283e	3.13d
Pooled SE		0.854	0.449	0.659	1.519
Two-way ANOVA		<i>P</i> value			
Agricultural wastes (AW)		0.0001	0.0001	0.0001	0.0001
Contact time		0.0001	0.0001	0.0001	0.0001
AW × contact time		0.0001	0.0001	0.0001	0.0001

Means followed by the same letter are not significantly different

Metal concentrations in whole-fish body were higher than those of HM-polluted water. This is because fish accumulated more metals in their bodies. Similar results were obtained by Abdel-Tawwab et al. (2013, 2016). Khalil and Hussein (1997) reared Nile tilapia for 9 months in secondary effluents with 1.0 Pb mg/L and found that the concentration of Pb in fish flesh was tenfold higher than the food standard. It is also noticed that HM accumulation in fish body increased as contact time increased. Easa et al. (1995), Shereif et al. (1995) and Shereif and Mancy (1995) studied Pb, Cu, Zn, and Cd in Nile tilapia reared in fishponds received wastewater. They found that HM concentrations in the fish increased by increasing the culture period.

## Conclusions

Rice straw, sugarcane bagasse, and maize stalks are effective and cheap adsorbents for removal of Pb, Cd, Cu, and Zn from polluted water. They also reduced HM bioaccumulation in fish body. Rice straw showed higher adsorption efficiency than the others.

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