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Bioaccumulation of industrial heavy metals in rats and bioremediation using calcium alginate.

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Abstract: Evaluation of industrial effluents poured in drains connected to agricultural canals was achieved through estimating heavy metal concentrations. The recorded metals were arranged descendingly as Mn, Fe, Zn, Cd, Co, Ni, Pb, Cu, and Cr in effluent samples. Treatment of effluent samples with Ca-alginate for 5 hours exhibited a significant decrease in metal concentration compared to untreated effluents. By exposing young male rats to different effluent concentrations (10%, 50% and 100%) and treated 100% effluent group for 11 weeks, metals effectively accumulated in the liver of young rats, depending on the effluent concentration. While the treated group with Ca-alginate showed significant amelioration in metal bioaccumulation in liver. Biochemical studies on young rats exhibited significant disturbance in liver oxidant/antioxidant parameters according to the effluent concentrations in all effluent groups. The antioxidants, superoxide dismutase, catalase, glutathione-S-transferase levels and glutathione content were significantly decreased in effluent groups compared to control. This was accompanied by significant elevation of malondialdehyde and protein carbonyl, as oxidative stress markers. However, all these changes were ameliorated in treated group. Therefore, Ca-alginate can be effectively used for the remedy of heavy metals in industrial effluents and maintain animal health.

keywords: Industrial effluents, heavy metals, liver, bio remedy.

1.Introduction

The scarcity of fresh water is a major challenge in Egypt that goes with the increase in population growth [1]. Water resources used for agricultural activities may be of poor-quality resulting from either natural or anthropogenic contamination or even both [2].

The direct disposal of effluents from various industries in a nearby water body without any treatment is a common practice in several countries including Egypt. Most industries do not have a plan for effluent treatment, and even if they have, they are not implemented because of the cost [3,4].

Unfortunately, the disposed effluents in drains are connected to several canals used for agricultural activities, either for irrigation or watering livestock [5]. Measurements of heavy metals (Cu, Co, Cr, Fe, Mn, Cd, Pb, Ni and Zn) are necessary to evaluate the healthiness of water to be suitable for irrigation and/or drinking [6,7]. Among various contaminants, heavy metals are known to have slow degradation, and high potential for accumulation and biomagnification causing toxicity through food chains [8].

In Mansoura city, Mansoura Company for Resins and Chemicals Industry (MRI) is a pioneer in the production of formaldehyde, urea, phenolic resins including molding powder, brake lining, foundry resins and other chemicals in Egypt, the Middle East and Africa [9]. The illegal discharge of large amounts of untreated effluents directly to the nearest water body represents an environmental problem. Several farmers ignore the deleterious effects of these xenobiotics, where water mixed with effluent components may be used for agricultural activities.

The assessment of systemic toxicity of untreated MRI effluents using a mammalian test model will be more relevant to the health of livestock. Biomarkers of systemic toxicity in mammals depend mostly on oxidants/antioxidants analyses to clarify the possible mechanisms of toxicity and tissue damage after exposure to a mixture of xenobiotics [10,11]. The liver is the major organ for conjugating and detoxifying any potential toxic substances. It is also essential for intermediary metabolism and the synthesis of several important compounds [12]. Several studies have reported that intracellular accumulation of metals stimulates free-radical reactions causing continuous production of reactive oxygen species (ROS). These results are in an imbalance between oxidant and antioxidants leading to lipid peroxidation and protein modification [11,13 &14].

On the other hand, bioremediation of effluent water has become a great concern [15]. Biosorbents, especially those derived from seaweeds (macroscopic algae) and alginate derivatives are widely abundant and less expensive than industrial synthetic adsorbents [16]. Algins are salts of alginic acid, a natural polymer found in brown algae (*Phaeophyceae)*. This polymer is extracted by treating seaweeds with sodium carbonate solution, where alginic acid is precipitated and sodium salt is formed. Alginate products are known to have high sorption uptake, abundance, high selectivity, low cost and no toxic effects [17]. Therefore, this study aims to evaluate the quality of MRI effluents through an in vivo experimental study, designed to mimic the exposure of livestock in rural areas that receive MRI effluents at different concentrations. The study is also extended to use alginate beads in the treatment of effluent water and examine their effects on metal bioaccumulation and oxidative stress markers in young male rats.

2. Materials and methods

2.1. Ecological studies

2.1.1. Study area

The present study was carried out at the center of Dakahlia Province, precisely near the Mansoura Company for Resins & Chemicals Industry (MRI). This factory is located at Sandoub-Elsinbellaween road in Mansoura city (31°05'00" N, 32°0'48" E), approximately 40 km from Damietta port. The company pours untreated effluents directly into El-Mansoura drain that passes through several agricultural villages such as Ezbet Shawwa, Sandoub, Telbana, Miniet Sandoub, Belgai, Ezbet Belgai, and Gemiza Belgai.

2.1.2. Effluent sampling

Water samples were collected directly from the discharge point of the effluents of the MRI company, during the period from February to April 2020 (3 samples/month). The samples were taken in clean polyethylene bottles of 10 liter capacity, where each bottle was rinsed three times before collecting the sample. 5ml of concentrated nitric acid was added to the whole sample to prevent heavy metals from precipitating.

2.1.3. Heavy metals analysis

The samples were analyzed for different heavy metal concentrations (Cu, Co, Cr, Fe, Mn, Cd, Pb, Ni and Zn) and the concentrations were recorded on a Buck Scientific Accusys 211 series “Atomic Absorption Spectrophotometer”, USA, by an air/acetylene flame system.

2.1.4. Effluents treatment

2.1.4.1. Preparation of Ca-alginate beads

According to EL-Tayieb and others (2013), a 4% w/v sodium alginate solution (cross-linker solution) was prepared by mixing fine sodium alginate powder with distilled water while stirring [18]. The latter solution was added dropwise into 100 ml of 3% $CaCl_2$ solution at 25˚C. Ca-alginate hydrogel spheres were formed into equal sized beads upon contact with the cross-linker solution and were left overnight to stabilize. The formed beads were washed several times with bi distilled water. Beads were filtered and left to dry to be ready for use.

2.1.4.2. Application of alginate sorbents on effluent samples

Sorption was performed by adding 100g dried Ca-alginate beads to a volume of 1000 ml effluent sample in a reactor. Sorption was evolved with the mixture under orbital shaking at 25°C. After 5 hours, volume samples were collected from the reactor and metal concentrations were detected again by atomic absorption spectroscopy, USA by an air/acetylene flame system.

2.2. Animal experimental design

A total of 50 young male Wistar rats (45±5 g) were obtained from the Egyptian Institute for Serological and Vaccine Production, Helwan, Egypt. The rats were allowed to acclimatize for one week to standard conditions of illumination with a 12 hrs light and dark cycle at 23±3°C room temperature and 40±5% humidity. Water and food were consumed *ad libitum* according to the Ethics Committee of Mansoura University, Egypt. The food consisted of corn, soybean pulp, sunflower seed meal, alfalfa pellets, molasses, vitamins, and minerals that are commonly used for poultry farms.

After the acclimation period, rats were randomly divided into five groups (10 rats/each) as follows:

Group 1 was the control (CN) group, which received tap water.

Group 2 received 10% concentration of MRI effluents (10%.EF).

Group 3 received 50% concentration of MRI effluents (50%.EF).

Group 4 received 100% concentration of MRI effluents (100%.EF).

Group 5 received 100% effluents after treatment with Ca-alginate beads (100%.EF+ AG) group.

Each group received specific drinking water daily for 11 weeks.

2.2.1. Animal investigations

2.2.1.1. Tissue sampling

Immediately after collecting blood samples, rats of each group were dissected, and the thyroid gland and a part of the right lobe of the liver were separated, for histological studies. However, approximately 0.5 g from the left lobe of liver was taken, homogenized in 5 ml cooled distilled water, and centrifuged at 860 Xg for 15 minutes. The clear supernatant was collected and stored at -20°C until analysis of antioxidants and oxidative stress markers. A third sample of the liver tissue was kept at -20°C for heavy metal analysis

2.2.1.2. Heavy metal concentrations in liver

Nitric-sulfuric-perchloric acid digestion is an approach that is partly modified from that of Tinggi *et al*. [19]. One gram of each sample was weighed into a conical flask, and 10 ml of concentrated HNO_3 was added. The conical flask was allowed to heat on a hot plate at 95°C until white fumes evolved and then left to cool at room temperature. After cooling, 3 ml of concentrated H_2SO_4 was added and then heated until the release of orange fumes. The flask was allowed to cool again at room temperature, and then 2 ml of $HClO_4$ was added and heated until the solution was reduced to approximately 5 ml. Each digested sample was filtered through 0.45-µ Millipore membrane filter paper diluted to 50 ml with deionized water in a volumetric flask and analyzed using a Buck Scientific atomic absorption spectrometer (model accusys 211, USA) equipped with an air/acetylene flame and hollow cathode lamps for the analyzed elements.

2.2.1.3. Liver antioxidants and oxidative stress markers

The liver superoxide dismutase (SOD) and catalase (CAT) activities, in addition to reduced glutathione (GSH) content and malondialdehyde (MDA) concentration were estimated using the specific enclosed methods of Biodiagnostic Co. Dokki, Giza, Egypt. However, an ELISA kit was used to determine glutathione S-transferase (GST) concentration in the liver according to the manufacturer's protocol of Cusabio, USA. Moreover, the protein carbonyl (PC) level in the liver was assessed according to (Pedemonte *et al*. (2015) the manufacturer's protocol enclosed in the Cayman Chemical Kit (Michigan, USA) [20].

Statistical analysis

The data were analyzed using the GraphPad Prism software program (v 5.04 GraphPad Software Inc., La Jolla, CA) using one-way ANOVA. All the results were recorded as the mean ± SD, where statistically significant data were considered at $p < 0.05$.

3. Results

3.1. Ecological studies

3.1.1. Heavy metals concentration in the effluent samples

Fig. (1) illustrates the mean concentrations of heavy metals (Cu, Co, Cr, Fe, Mn, Cd, Pb, Ni and Zn) in MRI effluents (EF) and effluents treated with alginate (EF+AG). The mean concentrations of heavy metals in EF samples were arranged descendingly as Mn, Fe, Zn, Cd, Co, Ni, Pb, Cu, and Cr, but after treatment with Ca-alginate they were Mn, Fe, Zn, Cd, Pb, Ni, Co, Cu, and Cr. Most of the detected heavy metals in MRI effluent water exceeded the WHO limits for drinking water, where Co (0.139±0.015) mg/L, Fe (1.235±0.520) mg/L, Mn (1.681±0.496) mg/L, Cd (0.247±0.071) mg/L, Pb (0.120±0.008) mg/L, Ni (0.122±0.034) mg/L and Zn (0.945±0.113) mg/L compared to (0.04), (0.3), (0.4), (0.003), (0.01),(0.07) and (0.2) mg/L WHO limits, respectively. However, according to FAO irrigation water limits, MRI effluents showed unsuitable concentrations of Co, Mn and Cd compared to the (0.05), (0.2) and (0.01) mg/L FAO limits. On the other hand, treatment of MRI effluent water with alginate showed a significant decrease in all the detected heavy metals compared to the untreated effluent water. Although this decrease was not below standard limits, it was greatly near their values in most of the investigated metals.

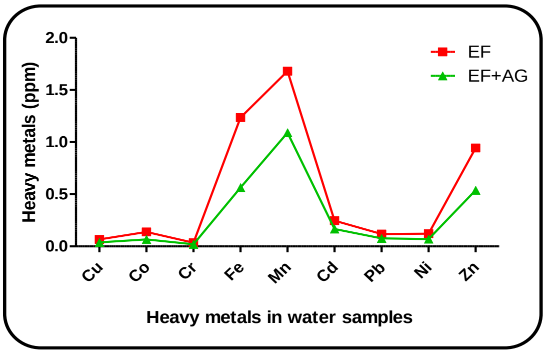

Fig.1. Mean values of heavy metal concentrations (ppm) of effluent samples

3.2. Animal studies

3.2.1. Heavy metal concentrations in the liver of the different investigated groups

The obtained data in Table 1 show a significant increase in the accumulation of heavy metals (Cu, Co, Cr, Fe, Mn, Cd, Pb, Ni and Zn) in the liver of MRI effluent groups dependent on effluent concentration compared to the control (CN) group. However, Co and Cr in 50%.EF group in addition to Mn in 10%.EF group were nonsignificant compared to CN group. The metal concentration was significantly decreased with 100%EF treatment by Ca-alginate. This reduction was significant in all detected metals compared to 100%EF group, and mostly with 10% & 50% EF groups. The accumulation of heavy metals in the liver tissues of the investigated groups was descendingly arranged in the following order: (Mn, Co, Cu, Cd, Fe, Pb, Zn, Cr, Ni) for the CN group, (Fe, Mn, Zn, Pb, Cd, Cu, Co, Ni, Cr) for 10%EF group, (Fe, Mn, Zn, Pb, Cd, Co, Cu, Ni, Cr) for 50%EF group, (Fe, Mn, Zn, Pb, Cd, Co, Cu, Cr, Ni) for 100%EF group and (Mn, Zn, Cd, Fe, Co, Pb, Cu, Ni, Cr) for 100%.EF+AG group. Moreover, by comparing 100%.EF+AG group to CN one, heavy metals concentration showed nonsignificant difference except (Cd) showed significant increase compared to CN.

Table 1. Heavy metal concentrations in the livers of the control and different investigated rat groups.

Groups / Metals(ppm)	CN	10%EF	50%EF	100%EF	100%EF+AG
Cu	0.005±0.001	0.0111 ^{ab}±0.003	0.0115 ^{ab}±0.0019	0.0261 ^{ab}±0.003	0.003±0.005
Co	0.0066±0.0007	0.0103±0.003	0.0123±0.003	0.0353 ^{ab}±0.004	0.0047±0.004
Cr	0.00031±0.0001	0.0022±0.0002	0.0028±0.0009	0.0086 ^{ab}±0.0043	0.00033±0.0001
Fe	0.0013±0.0003	0.0823 ^{ab}±0.011	0.125 ^{ab}±0.041	0.143 ^{ab}±0.021	0.0077±0.003
Mn	0.0148±0.0007	0.0512±0.007	0.084 ^{ab}±0.28	0.090 ^{ab}±0.015	0.044±0.005
Cd	0.0016±0.0003	0.0131 ^{a}±0.0026	0.018 ^{ab}±0.002	0.037 ^{ab}±0.004	0.0096 ^{a}±0.0006
Pb	0.0012±0.0001	0.016 ^{ab}±0.005	0.0196 ^{ab}±0.003	0.042 ^{ab}±0.007	0.003±0.0002
Ni	0.0003±0.0001	0.0034^{ab}±0.0008	0.0037^{ab}±0.0009	0.0076 ^{ab}±0.002	0.0005±0.0001
Zn	0.0011±0.0006	0.0281 ^{a}±0.0028	0.0378 ^{ab}±0.013	0.050 ^{ab}±0.006	0.0125±0.0009

(Data are presented as the mean ± SD.) CN=Control tap water; 10%, 50% & 100%.EF=concentrations of MRI effluents; 100%.EF+AG =100% effluents treated with Ca-alginate

a: significant change in comparing different groups with the CN group ($p< 0.05$)
b: significant change in comparing EF groups with 100%.EF+AG group ($p< 0.05$)

3.2.2. Liver antioxidants and oxidative stress markers

The obtained data showed obviously decreased liver antioxidant activities of CAT, SOD, GST, and GSH content with increasing concentrations of MRI effluents compared to the control group. This decrease was mostly significant with 50%.EF and 100%.EF groups. On the other hand, oxidative stress markers (MDA and PC) showed significant increase with drinking different concentrations of MRI effluent groups compared to the control group. Treatment with Ca-alginate exhibited a significant increase in the studied antioxidants in the liver of 100%.EF+AG rat group which also exhibited a significant decrease in MDA and PC compared to the 50%EF and 100%.EF groups (Fig. 2, 3, 4, 5, 6, 7).

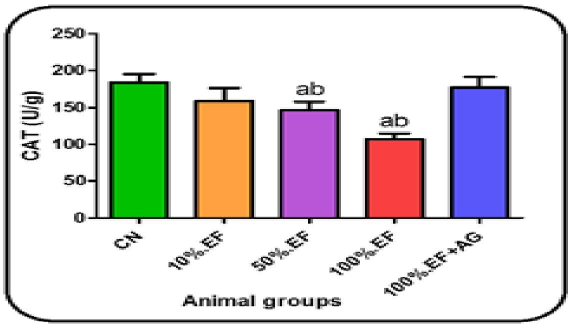

Fig. 2. Catalase (CAT) activity (U/g) in the control and different investigated rat groups.

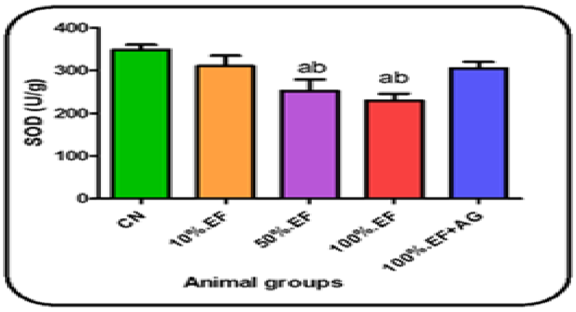

Fig. 3. Superoxide dismutase (SOD) activity (U/g) in the control and different investigated rat groups.

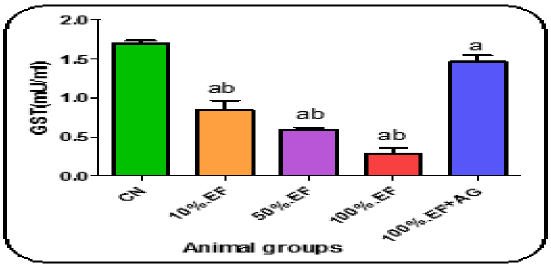

Fig. 4. Glutathione S transferase (GST) activity (mU/ml) in the control and different investigated rat groups.

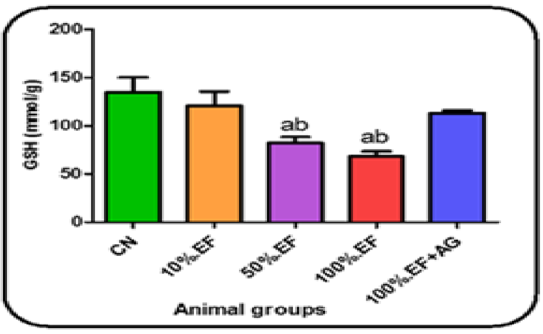

Fig.5. Reduced glutathione (GSH) content (mmol/g) in the control and different investigated rat groups.

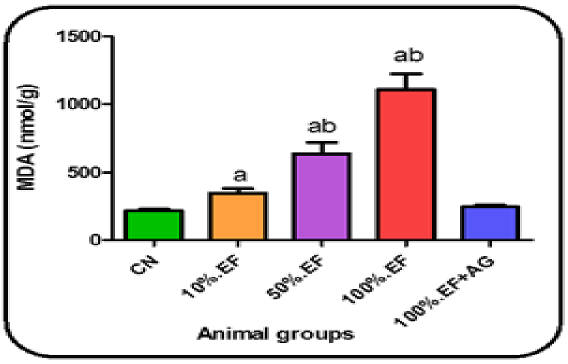

Fig. 6. Malondialdehyde (MDA) concentration (nmol/g) in the control and different investigated rat groups.

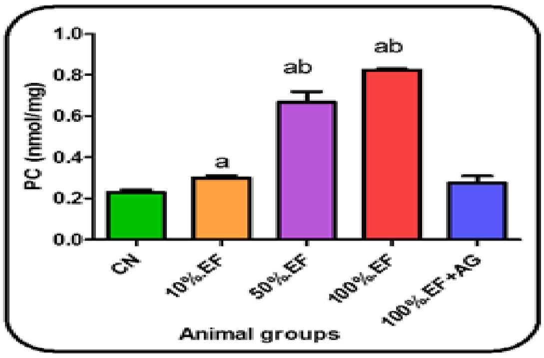

Fig.7. Protein carbonyl (PC) level (nmol/mg) in the control and different investigated rat groups.

4. Discussion

4.1. Ecological studies

The direct discharge of untreated industrial effluents into drains, canals, or rivers worsens the problem of water pollution. The suitability of water used from drains, canals for irrigation purposes and watering livestock was taken into consideration in this study. Actually, this was achieved in the present study where data showed an increase in heavy metals (Co, Fe, Mn, Cd, Pb, Ni and Zn) in MRI effluent samples above WHO limits. This may be because the MRI company is famous for producing foundry resins. The process of manufacturing these resins passes through different steps and reactions in which salts of divalent ions of Mn, Zn, Cd, Co, Fe, Ni and Pb are used as catalysts [21].

Bioremediation of MRI effluent samples using Ca-alginate derived from brown algae was achieved in this study. The utilization of algae as biosorbents is distinguished by unproduced toxic substances such as bacteria or fungi [22]. The obtained data showed that the treatment of effluent samples for 5 hrs using Ca-alginate beads showed a significant decrease in heavy metal concentrations compared to untreated effluents. Zabochnicka-Swiatek and Krzywonos (2014) explained the mechanism of metal biosorption by microorganisms as the macromolecules of the cell wall structure, such as polysaccharides and proteins, have a negatively charged functional groups such as carboxyl, sulfhydryl, carbonyl, and hydroxy groups [23]. These functional groups could gravitate positively charged metals found in a solution through the adsorption process [24].

4.2. Heavy metals accumulation and biochemical studies

In a process mimicking young livestock exposed to different concentrations of MRI effluents at different sites, the present study was concerned with possible hazards in young rats that received different effluent concentrations daily for 11 weeks. Metal bioaccumulation in the rat liver is of great concern due to its high sensitivity in the mammalian system to chemical toxicity. The obtained results showed that Mn, Fe, Zn, Cd and Pb were the most accumulated metals in the liver depending on the effluent concentration (10%, 50% and 100%) compared to the control group. This may be due to the absorbance of metals by intestinal cells that pass through the blood to the liver for the detoxification process by metallothionein protein synthesized in the liver [10]. On the other hand, the increased heavy metals of MRI effluents could be a source of reactive oxygen species (ROS) that might disturb the oxidant/antioxidant defense system in the body. This was clearly observed in this study through the significantly elevated levels of liver MDA and PC in all effluent groups referring to lipid peroxidation and protein oxidation in liver cells. These results are in agreement with where extreme generation of free radicals after exposure to Cd was the main cause of MDA and PC increases [16]. In parallel, the obtained data showed a significant decrease in the liver SOD, CAT, and GST activities and GSH content in the effluent groups compared to the control group. The antioxidants SOD and CAT are the first line for body defense against toxicity. These enzymes were reported to be decreased due to excessive production of ROS induced by metal exposure [1]. explained this through an in vivo study, where Pb and Cd can be combined with reductive sulfhydryl groups to antagonize the reducing effect of SOD and GSH and weaken the ability of organisms to oxidize and metabolize lipid products, leading to enhanced lipid peroxidation [25]. (2021) revealed that exposure to Cd could knock out the metallothionein gene which resulted in decreased CAT activity [11]. Furthermore, in the literature, there are many conflicting reports on the effect of metals on the activity of GST. However, (2015) attributed the decrease in GST to exposure to heavy metals such as Cd which leads to the formation of transition complexes with protein enzymes and consequently inhibits the activity of antioxidant enzymes [26].

Bioremediation using algae has been successfully used as a biosorbent for heavy metal detoxification [16,22]. The treatment of 100%EF with Ca-alginate (100%EF+AG) group showed significantly decreased accumulation of the studied heavy metals in the rat liver compared to the untreated 100%.EF group. This result exactly referred to the successful role of Ca-alginate as a biosorbent for heavy metal detoxification. This was significantly clear with decreased oxidative stress parameters (MDA and PC) and increased antioxidants (SOD, CAT, GSH, GST). attributed this improvement to the marked reduction in metallothionein levels after treatment of heavy metal polluted water with algae [16].

5. Conclusion

In conclusion, this study illustrated the heavy metal concentrations in MRI untreated effluents. Treatment of effluents by using Ca-alginate represents an effective and safe biosorbent for heavy metal decontamination and purification of effluent water. In a process mimicking livestock exposure to polluted effluents at different sites, young rats exposed to different effluent concentrations showed significant reduction in antioxidants accompanied with significant increase in oxidative stress markers that may affect animal health and human health through the food chain. This may indicate that closer villages to the MRI company are at greater risk.

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