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Araştırma Makalesi / *Research Paper*

Removal of Heavy Metals (Copper and Lead) Using Waste Eggshell with Two Different Species and Three Different Forms

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ABSTRACT

Since copper and lead are the most well-known heavy metals, eggshells were used to remove them from the aqueous solution. In this study, it was used two species; Quail (*Coturnix coturnix japonica*) and Greylag Goose (*Anser anser*) and three forms (pure, powdered and calcined forms) of eggshells. Using coupled plasma optical emission spectroscopy (ICP-OES), Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) methods, it was investigated whether copper and lead were adsorbed on eggshells; in different species and different forms. According to ICP-OES results, it was observed that calcined eggshells retained more amount of Cu and Pb than uncalcined eggshells. By analyzing the FTIR results, even if the eggshells were in different forms, the characteristic bands of the eggshells were almost seen in samples. After the adsorption process, new bands arose in addition to the characteristic eggshell bands. These new bands are thought to be related to the Cu and Pb loading in the eggshell. With SEM images, it was observed that metal loaded accumulated on the outer surface of the eggshell. This result is also in good agreement with the EDS results.

Keywords: Copper, eggshell, lead, low-cost adsorbent

İki Farklı Tür ve Üç Farklı Formda Atık Yumurta Kabuğu Kullanılarak Ağır Metallerin (Bakır ve Kurşun) Uzaklaştırılması

ÖZ

Bakır ve kurşun en iyi bilinen ağır metaller olduğundan, bunların sulu çözeltiden uzaklaştırmak için yumurta kabukları kullanıldı. Bu çalışmada iki tür kullanıldı; bıldırcın (*Coturnix coturnix japonica*) ve boz kaz (*Anser anser*) ve yumurta kabuğunun üç formunu (saf, toz ve kalsine formlar) kullandık. Eşleştirilmiş plazma optik emisyon spektroskopisi (ICP-OES), Fourier-dönüşümlü kızılötesi (FTIR) spektroskopisi, taramalı elektron mikroskobu (SEM) ve enerji dağılımlı spektroskopi (EDS) yöntemleri kullanılarak, yumurta kabuklarına bakır ve kurşunun adsorbe edilip edilmediği araştırıldı; farklı türlerde ve farklı formlardaki yumurta kabuklarında. ICP-OES sonuçlarına göre, kalsine edilmiş yumurta kabuklarının, kalsine edilmemiş yumurta kabuklarından daha fazla miktarda Cu ve Pb tuttuğu gözlemlenmiştir. FTIR sonuçları analiz edildiğinde, yumurta kabukları farklı formlarda olsa bile, örneklerde yumurta kabuklarının karakteristik bantları hemen hemen aynı bölgelerde görülmüştür. Adsorpsiyon işleminden sonra karakteristik yumurta kabuğu bantlarına ek olarak yeni bantlar ortaya çıkmıştır. Bu yeni bantların yumurta kabuğundaki Cu ve Pb yüklü olmasıyla ilgili olduğu düşünülmektedir. SEM görüntüleri ile yumurta kabuğunun dış yüzeyinde metalin biriktiği gözlenmiştir. Bu sonuç, EDS sonuçlarıyla da iyi bir uyum içindedir.

Anahtar Kelimeler: Bakır, yumurta kabuğu, kurşun, ucuz adsorbent

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INTRODUCTION

Environmental pollution significantly affects the health of living things. Heavy metals, which have the feature of accumulating in nature because they are not biodegradable, are dangerous for living things when they exceed certain limits (Veli and Alyuz, 2007). Adsorption is an easy and inexpensive method for removing heavy metals from aqueous solutions. The adsorption process takes a very short time and is the most preferred method to remove heavy metals by adsorption due to its ease of use and simplicity (Alaba et al., 2018; Musonge and Harripersadth, 2021). Biosorbents are used effectively to remove heavy metals from the environment (Yang et al., 2021). In addition, biosorbents do not cause additional harm to the environment and do not release substances that may be harmful to the environment. Egg is one of the most used, easily accessible, cheap and basic food sources in daily life. Since their shells are sometimes rich in calcium content, they are used in the pharmaceutical and food industry as a source of calcium (Waheed et al., 2019), as a bio-ceramic (Tangboriboon et al., 2019), composite (Feng et al., 2014), fertilizer (King'Ori, 2011) and biosorbent (Podstawczyk et al., 2014), while they are often thrown away, especially in household use. However, the physical and chemical structures of the eggshells are very suitable as biosorbents. Specially, porous structure and electronegativity due to $CaCO_3$ in its chemical content are desirable and preferred adsorbate property for adsorption (Wang et al., 2018; Kaya Kınaytürk et al., 2021). Adsorption process for removing heavy metals from the environment is one of the most preferred processes due to its strong affinity and high loading capacity. Moreover, they are cost-free as they are waste materials (Musonge and Harripersadth, 2021). Since they have gotten porous and layer-by-layer structure and they can adsorb heavy metals, or they can trap many pollutants, dyes and organics. In addition, the calcined state can absorb much more amount of heavy metals than its natural state (Park et al., 2007). Pb^{2+} is considered a potential carcinogen and associated with the cause of many diseases, especially cardiovascular, kidney, blood, nerve and bone diseases. Cu^{2+} is an essential element, but its high concentrations in food and fodder crops are great concern due to increased toxicity to humans and animals (Hashmi et al., 2013). It is important to remove heavy metals such as copper and lead from the environment before reaching irrigation water in agriculture. At the same time, removing heavy metals from drinking water can prevent living things from damaging organs in their bodies. Therefore, many researchers have proposed various methods to purify heavy metals from aqueous solutions (Gebru and Das, 2017; Yu et al., 2021). Some of these are chemical precipitation, coagulation, ion exchange, solvent extraction, filtration, evaporation and membrane methods (Park et al., 2007). The disadvantage is that most of these methods require a few pre-processes and additional operations. In this study, eggshell residue was used as a biosorbent material. The aim is to remove Pb^{2+} and Cu^{2+} from the aqueous solution. At the same time, we recycle the waste eggshell. We used two types of eggshells in three different forms. Types (species) of eggshells were Quail (*Coturnix coturnix japonica* (*CCJ)* and Greylag Goose *(Anser anser (AA)*). Three different forms were 1-pure eggshell, 2-powdered eggshell, 3-calcined eggshell. We used $CuCl_2$ and $Pb(NO_3)_2$ as metal salts in the experiments. After preparation in aqueous solutions of copper and lead heavy metal salts, we examined the adsorption phenomenon for each sample in the respective eggshell type and form. Abbreviations are made as follows:

CCJ: pure quail eggshell
AA: pure grey goose eggshell
C-CCJ: calcined quail eggshell
C-AA: calcined grey goose eggshell
Cu@CCJ: CCJ treated with $CuCl_2$
Pb@CCJ: CCJ treated with $Pb(NO_3)$
Cu@C-CCJ: C-CCJ treated with $CuCl_2$
Pb@C-CCJ: C-CCJ treated with $Pb(NO_3)_2$
Cu@AA: AA with treated $CuCl_2$
Pb@AA: AA with treated $Pb(NO_3)_2$
Cu@C-AA: C-AA with treated $CuCl_2$
Pb@C-AA: C-AA with treated $Pb(NO_3)_2$

MATERIALS AND METHODS

Materials and Chemicals

Eggshell samples *CCJ* and *AA* were obtained from Isparta University of Applied Sciences, Education Research and Application Farm. Eggshell samples were taken from incubation wastes of this farm. The compounds of $Pb(NO_3)_2$ and $CuCl_2$ in solid form were purchased from Sigma-Aldrich chemical company (USA) and they were used without further purification.

Experimental

The cleaning procedure in our previous article was applied to *CCJ* and *AA* eggshells (Kaya Kınaytürk et al., 2021). We used samples in three different forms: shell form, powdered form, calcined form. Shell form needed no further process on it. For powdered form we ground samples in an agate mortar, for calcined form, this part was calcined at 900C° for 2 hours. The schematic representation and photographs were shown in Figure 1.

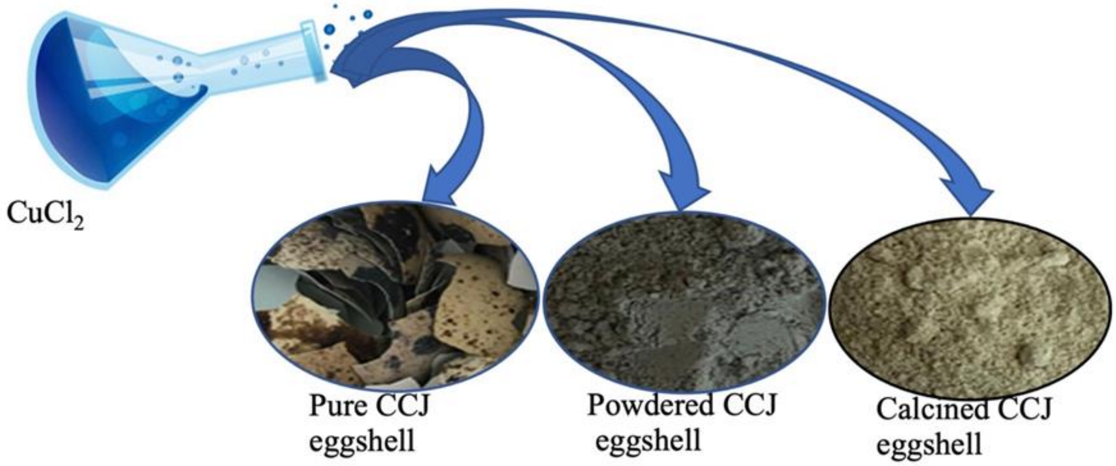


Figure 1. Pure, powdered and calcined CCJ of eggshells treated with $CuCl_2$

0.05 M, 100 mL $Pb(NO_3)_2$ and $CuCl_2$ solutions were prepared with ultra-distilled (18,2 Ω) water. 1 g of each eggshells samples were mixed with 0.05 M, 100 mL $Pb(NO_3)_2$ and $CuCl_2$ solutions in an ultrasonic bath. After that, they were shaken from time to time and adsorption was applied for 48 hours. Filter and dried eggshells samples were analyzed by FTIR spectroscopy, ICP-OES and SEM. In addition to that, to investigate the differences we used all analytical techniques for pure eggshell samples.

Before ICP-OES analyses, samples were prepared as follows: 0,25 g sample + 4 mL 65% HNO_3 + 2 mL 40 % HF + 2 mL 98% H_2SO_4 and Milestone Start D model microwave digestion system used, with Perkin Elmer ICP-OES The Optima 8000 in MAKÜ (Burdur Mehmet Akif Ersoy University), BILTEKMER (Scientific and Technology Application and Research Center) Burdur, Turkey. FTIR analyses were performed with Perkin Elmer Spectrum Fronter device using KBr pellet technique in the range of 4000-400 cm^{-1} in BILTEKMER. SEM and EDS analyses have been used to record the morphological data and the elemental composition of the samples with FEI Quanta FEG 250 in SDU (Süleyman Demirel University), YETEM (Innovative Technologies Application and Research Center), Isparta, Turkey.

RESULTS AND DISCUSSION

ICP-OES Results

The ICP-OES were used to determine the amount of heavy metal on eggshells after adding metallic aqueous solution on the uncalcined and calcined form of eggshells. They are also listed in Table 1 as mg/kg. In each type of eggshell, copper is less abundant on specimens. When looking at the same eggshell species, metals are more abundant in calcined eggshell form than uncalcined.

Table 1. Amounts of copper and lead on powdered and calcined form of eggshells

	Cu (mg/kg)		**Pb (mg/kg)**
Cu@CCJ	9920,729	**Pb@CCJ**	329827,916
Cu@C-CCJ	13496,012	**Pb@C-CCJ**	365295,926
Cu@AA	199743,213	**Pb@AA**	318076,028
Cu@C-AA	203998,447	**Pb@C-AA**	353325,416

When Table 1 is examined, it was seen that copper and lead heavy metals were adhering to the eggshell in each form. More heavy metals were retained in calcined eggshells than in uncalcined eggshells. It is thought that the reason of this increase was that when the eggshells were calcined, more heavy metal has adhered because the surface area increases (Park et al., 2007).

FTIR Spectroscopic Results

Since metal is adsorbed on the different forms of the eggshell, FTIR analysis can give an idea or clue about the location of the metal ion. Figure 2 and Table 2 show that the IR spectra and assignments list of CCJ, C-CCJ, Cu@CCJ, Pb@CCJ, Cu@C-CCJ, Pb@C-CCJ, respectively.

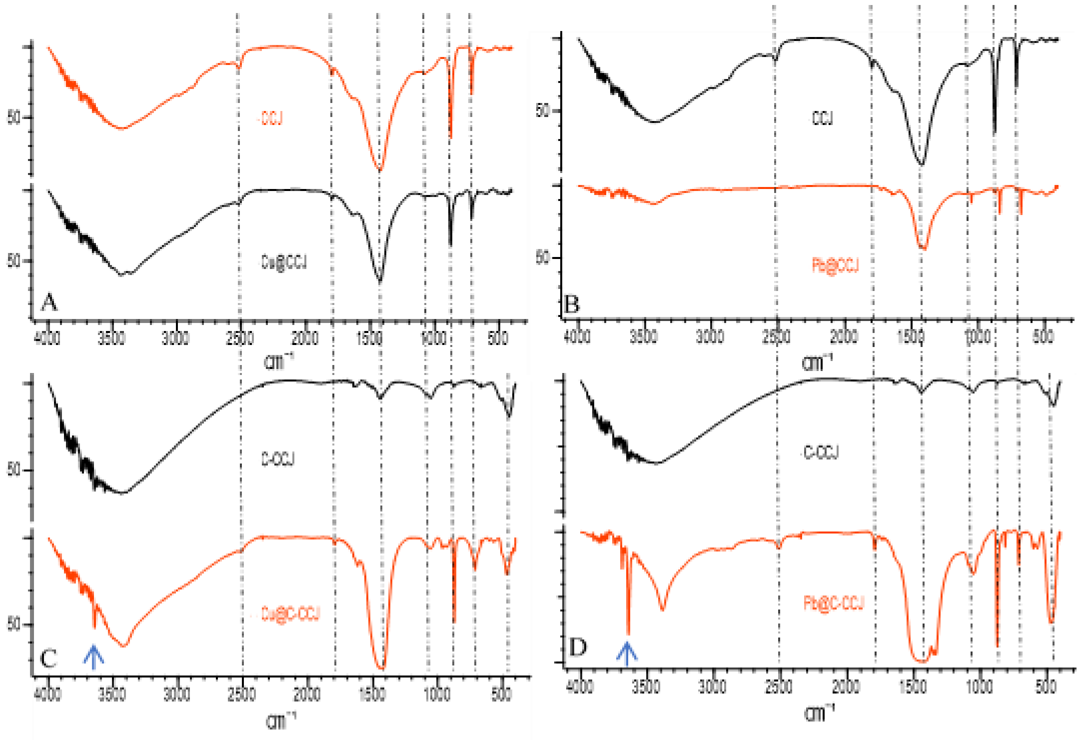


Figure 2. (A) CCJ and Cu@CCJ (B) CCJ and Pb@CCJ (C) C-CCJ and Cu@C-CCJ (D) C-CCJ and Pb@C-CCJ

Table 2. IR assignments of different forms of CCJ eggshell

Assignments	References	CCJ	Cu@CCJ	Pb@CCJ	C-CCJ	Cu@C-CCJ	Pb@C-CCJ
OH stretch	3688 (Park et al., 1988)						3690m
OH stretch	3643 (Naemchan et al., 2008)						3641s
CO_3^{-2} stretching	2516 (Kaya Kınaytürk et al., 2021)	2516 w	2516w	2516w	-	2515w	2511w
CO_3^{-2} stretching	1799 (Kaya Kınaytürk et al., 2021)	1799 w	1799w	1799vw	-	1797w	1794w
NO_2 stretching	1727 (Bhatia et al., 1983)			1728m			1735vw
Carbonyl group stretching	1645 (Tizo et al., 2018)	1631sh	1631sh	1638sh	1632sh	1617sh	1632sh
CO_3^{-2} stretching	1424 (Kaya Kınaytürk et al., 2021)	1424 s		1435s	1444m		1435s
C-O stretch	1408 (Basaleh et al., 2019)			1403s			1354s
CO_3^{-2} stretching	1082 (Kaya Kınaytürk et al., 2021)	1082 sh	1082sh	1105sh	1093sh-1056m	1093sh-1056m	1093sh-1051m
N-O stretching	1072 (Zhang et al., 2021)			1052m			
CO_3^{-2} in plane deformation	875 (Kaya Kınaytürk et al., 2021)	875 m		872s	876w	872s	873s
C-H out of plane	802 (de Luna et al., 2015)		822w	839m		-	814m
CCl_3 stretching	784 (Wallington et al., 2000)		788w			-	
CO_3^{-2} out of plane deformation	713 (Kaya Kınaytürk et al., 2021)	713 m	713m	713w	-	711m	711s
NO_3^- stretching	721 (Reddy et al., 2015)			679s			677s
Ca-O	447 (Pandit and Fulekar, 2017)				447m	468m	474s

s; strong, sh; shoulder, w; weak, m; medium means the intensity of the peaks

When Figure 2 and Figure 3 were examined, it wasn't clearly seen around 3640 cm^{-1} which belonged to OH stretching band in the uncalcined samples. It was observed after treated with heavy metals on calcined forms. This band was attributed OH^- band (Naemchan et al., 2008; Putra et al., 2017). While after calcination process, calcined eggshell shows the existence of OH in $Ca(OH)_2$ in the peak of around 3643 cm^{-1} (Naemchan et al., 2008). It was formed during adsorption of water by CaO (Renu et al., 2017). The bands around 3400–3600 cm^{-1} were caused by the vibration of water molecules in the uncalcined and calcined samples (Naemchan et al., 2008). 2516, 1799, 1424, 1082, 875 and 713 cm^{-1} are characteristic peaks of eggshells (Kaya Kınaytürk et al., 2021), (Anjaneyulu and Sasikumar, 2014; Awogbemi et al., 2020). One of the studies, the band at 2506 cm^{-1} was assigned to carbonate vibration (Tatzber et al., 2007). In this study, this band was seen at 2516 cm^{-1} that was observed at the same position, in our previous study (Kaya Kınaytürk et al., 2021). The band at 1799 cm^{-1} observed in pure eggshells disappeared after calcination but it was clearly visible after treatment with aqueous metal solution. The weak band around 1799 cm^{-1} and a shoulder around 1084 cm^{-1} have corresponded to C = O bonds related to carbonate and the symmetric stretching of CO_3, respectively (Kazemi et al., 2017; Queiros et al., 2017). The band at around 1640 cm^{-1} can be attributed to the carbonyl group vibration (Tizo et al., 2018). The peak, which was the C-O stretching belongs to the symmetrical vibration of carbonate around 1424 cm^{-1}, was seen as the deepest peak in all eggshell forms (Eletta et al., 2016; Yusuff, 2017; Kaya Kınaytürk et al., 2021). The small bands around 874 and 712 cm^{-1} were attributed to the out-of-plane and in-plane deformation vibration bands of $CaCO_3$, respectively (Tsai et al., 2006; Kit, et al., 2020; Kaya Kınaytürk et al., 2021). In addition, generally sharp vibration band at 872 cm^{-1} was seen more deeply, especially in calcined form eggshell and treated with aqueous metal solution eggshell forms. This peak is evidence of the $CaCO_3$ in the eggshell structure.

When Figure 2(A) is investigated, the weak vibrational band at 788 cm^{-1} in Cu@CCJ was thought to arise as a result of the interaction of Cl between the surface of eggshell (Bae et al., 2006). The CH band at 802 cm^{-1} in the literature was seen as a weak band at 822 cm^{-1} in our study after treatment of copper aqueous solution (de Luna et al., 2015).

When Figure 2(B) is examined, a new weak band in Pb@CCJ was observed at 1728 cm^{-1}(N-O) which was thought to be caused by NO_2 (Bhatia et al., 1983). The shift of C–O peak from 1408 to 1403 cm^{-1} in the Pb@CCJ spectrum might be ascribed to the interaction between Pb(II) ions and carbonate group on CCJ surface (Basaleh et al., 2019). The CH band detected at 802 cm^{-1} in the literature, which was not observed in pure eggshell, was seen at 839 cm^{-1} after treatment with Pb aqueous solution (de Luna et al., 2015). A new medium band in Pb@CCJ was arise at 1052 cm^{-1} which was thought to be caused by NO stretching (Zhang et al., 2021). The CH and NO_3^- vibration bands were seen at 839 cm^{-1} (de Luna et al., 2015). and 679 cm^{-1} (Reddy et al., 2015), respectively.

When Figure 2(C) is investigated, characteristic bands of C-CCJ were seen at 1632, 1444, 1093, 1056, 876 and 447 cm^{-1} (Pandit and Fulekar, 2017). The vibration band at 447 cm^{-1} was thought to belong to CaO (Pandit and Fulekar, 2017). This band was shifted to 468 cm^{-1}in Cu@C-CCJ.

When Figure 2(D) is examined, the C-O peak at 1408 cm^{-1} in literature shifted to 1354 cm $^{-1}$ in Pb@C-CCJ. The C-O vibration band at 1056 cm^{-1} in C-CCJ was observed to shift to 1051 cm^{-1} in Pb@C-CCJ. The reason of this shift may be caused by the interaction of eggshells and Lead ions. The CaO vibration band at 447 cm^{-1} in C-CCJ shifted to 474 cm^{-1}. The reason of this shift was thought to be due to the interaction of the aqueous solution of the lead and the eggshells. In addition, new bands were seen at 1735 and 677 cm^{-1} in Pb@C-CCJ. These bands were attributed to result from NO_2 (Bhatia et al., 1983) and NO_3^- (Reddy et al., 2015), stretching, respectively. The CH band observed at 802 cm^{-1} in the literature was observed at 814 cm^{-1} in Pb@C-CCJ (de Luna et al., 2015).

Figure 3 and Table 3 show the IR spectra and assignments of AA, C-AA, Cu@AA, Pb@AA, Cu@C-AA, Pb@C-AA, respectively.

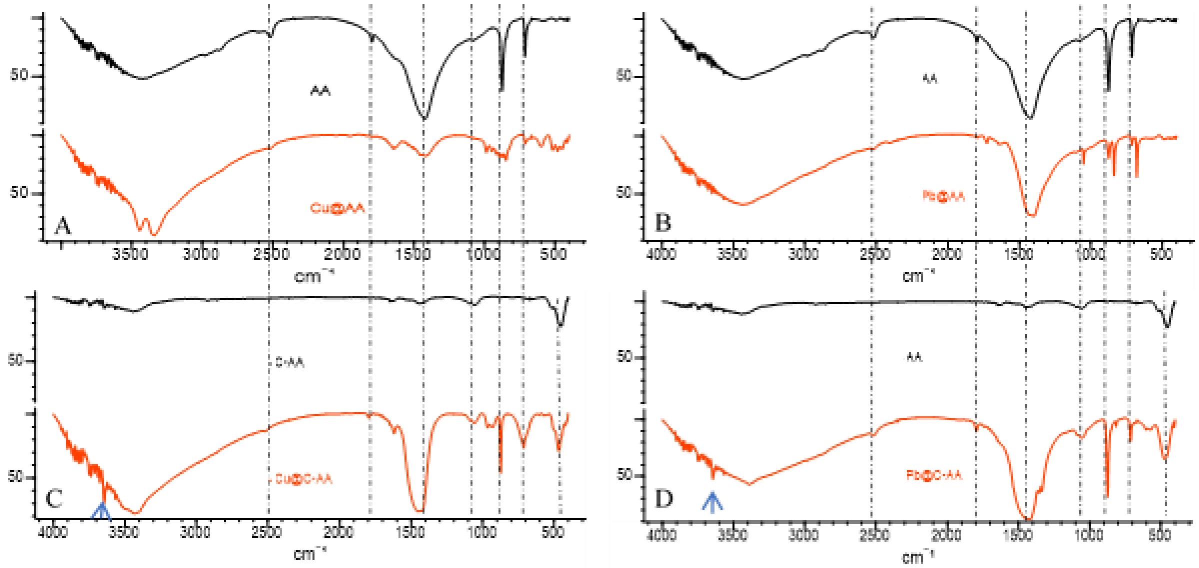


Figure 3. (A) AA and Cu@AA (B) AA and Pb@AA (C) C-AA and Cu@C-AA (D) C-AA and Pb@C-AA

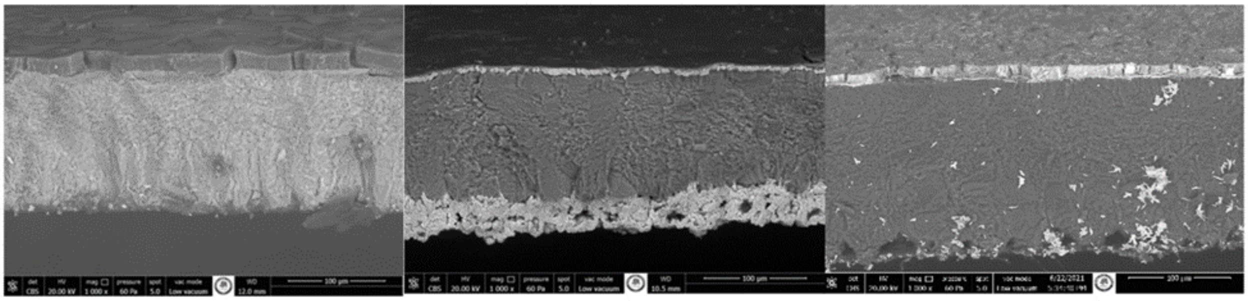
Table 3. IR assignments of different forms of AA eggshell

Assignments	References	AA	Cu@AA	Pb@AA	C-AA	Cu@C-AA	Pb@C-AA
OH	3643 (Naemchan et al., 2008)						3646m
CO_3^{-2} stretching	2516 (Kaya Kınaytürk et al., 2021)	2516m	2516w	2516w	-	2516w	2516w
CO_3^{-2} stretching	1799 (Kaya Kınaytürk, et al., 2021)	1800m	1800vw	1800w	-	1796w	1796w
NO_2 stching	1727 (Bhatia et al., 1983)			1729m			-
Carbonyl group stretching	1645 (Tizo et al., 2018)	1630sh	1645m	1645m	1635m	1621w	1629sh
CO_3^{-2} stretching	1424 (Kaya Kınaytürk et al., 2021)	1424s		1424s	1441m		1435s
C-O stretch	1408 (Basalehet al., 2019)			1397s			1356sh
CO_3^{-2} stretching	1082 (Kaya Kınaytürk et al., 2021)	1082sh	-	1104 sh	1091sh-1055m	1090sh-1056m	1092sh-1052m
N-O stretching	1072 (Zhang et al., 2021)			1051m			
CO_3^{-2} in plane deformation	875 (Kaya Kınaytürk et al., 2021)	874s	872s	874m	877w	873s	
C-H out of plane	802 (de Luna et al., 2015)		849w	837s			814m
CO_3^{-2} out of plane deformation	713 (Kaya Kınaytürk et al., 2021)	712s	712w	712w	-	713w	713m
NO_3^{-} stretch	721 (Reddy et al., 2015)			677m			-
Ca-O	447 (Pandit and Fulekar, 2017)				455s	467m	474s

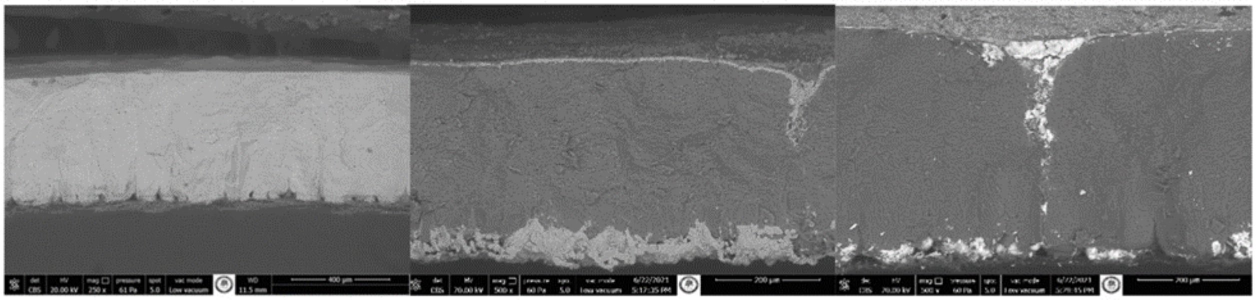
When Figure 3(A) was investigated, the peak at 849 cm^{-1} was attributed CH out of plane in Cu@AA, (de Luna et al., 2015). When Figure 3(B) was examined, the characteristic band of the C-O vibration at 1082 cm^{-1} in Pb@AA was seen to shift to 1104 cm^{-1}. The reason of this shift was thought due to the loaded of lead on the surface of eggshell. All other characteristic bands of eggshells were seen. In addition, bands at 1729, 1397,1051,837 and 677 cm^{-1} were thought that it might belong to the vibrations of NO_2 (Bhatia et al., 1983), CO (Basaleh et al., 2019), N-O (Zhang et al., 2021), CH (de Luna et al., 2015), NO_3^- (Reddy et al.,2015), respectively. When Figure 3(C) is investigated, characteristic bands for calcined goose eggshell; 1635, 1441, 1091, 1055 and 877 cm^{-1} were also observed. The vibration band at 455 cm^{-1} in the C-AA and thought to belong to Ca-O which was shifted to 467 cm^{-1} in Cu@C-AA. When we investigated the peaks in Figure 3(D), the shoulder band at 1356 cm^{-1} was attributed to C-O stretching in Pb@C-AA. In addition, newly band arose at 814 cm^{-1} in Pb@C-CCJ. This band was thought to result from C-H out of plane vibration (de Luna et al., 2015). The vibrational band of Ca-O at 455 cm^{-1} in C-AA was seen to be shifted to 474 cm^{-1} in Pb@C-AA (Pandit and Fulekar, 2017).

SEM-EDS Results

Figure 4 shows SEM images for eggshells in different forms. The SEM images were taken as a cross section. Thus, it was investigated whether the contents of the unpowdered eggshells and heavy metals were included. When Figure 4 is examined, bright parts show areas covered with metal in Figure 4A-C-E-F. In Figures 4A and D, the eggshells were seen as 4 layers.



(A)CCJ (B) Cu@CCJ (C) Pb@CCJ



(D) AA (E) Cu@AA (F) Pb@AA

Figure 4. SEM photographs of CCJ, Cu@CCJ, Pb@CCJ, AA, Cu@AA and Pb@AA

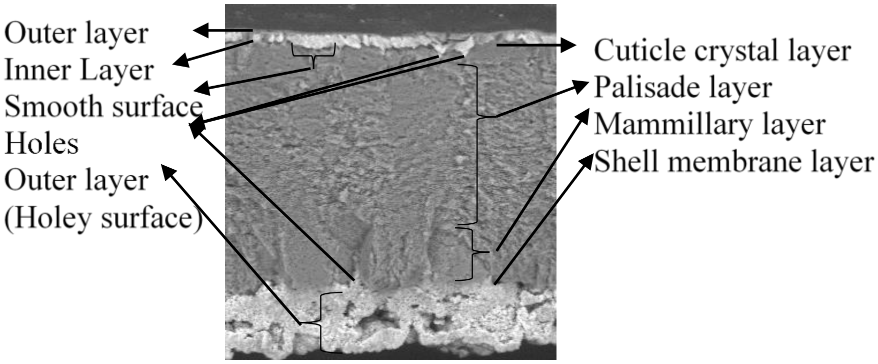
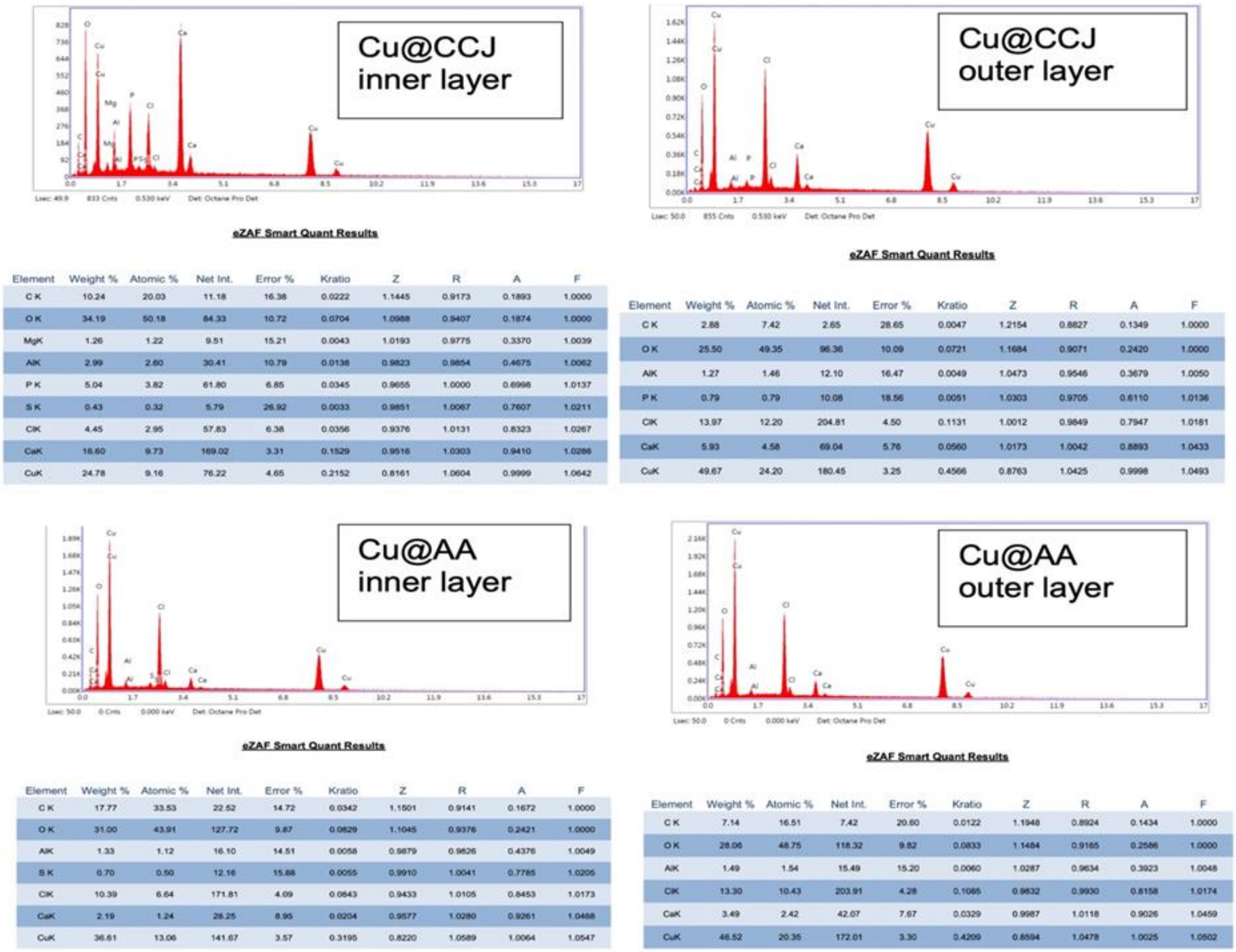


Figure 5. Eggshell layers

These layers are cuticle crystal layer, palisade layer, mammillary layer and shell membrane layer from top to bottom (Figure 5) (Hincke et al., 2008; Pérez-Huerta and Dauphin, 2016). Heavy metals were seen to accumulate on the outer surface of the eggshells. On the holey surfaces, it was observed that heavy metals penetrate inside, but unsurprisingly, heavy metals could not penetrate into the smooth surface of the eggshells. While the Cu residues in Figure 4B and E were 2 layers on the cuticle crystal layer of the eggshells, the Pb residues in Figure 4C and F were seen as a single layer accumulation. In Figure 4C, the bright parts in the palisade and mammillary layers are copper residues smeared from the outer part of the eggshell when taking SEM images. These results were also proven by the EDS results in Figure 6. The EDS results from inner and outer layers upon the cuticle crystal layers of Cu@CCJ and Cu@AA were shown in Figure 6.



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
C K	10.24	20.03	11.18	16.38	0.0222	1.1445	0.9173	0.1893	1.0000
O K	34.19	50.18	84.33	10.72	0.0704	1.0988	0.9407	0.1874	1.0000
MgK	1.26	1.22	9.51	15.21	0.0043	1.0193	0.9775	0.3370	1.0039
AlK	2.99	2.60	30.41	10.79	0.0138	0.9823	0.9854	0.4675	1.0062
P K	5.04	3.82	61.80	6.85	0.0345	0.9655	1.0000	0.6998	1.0137
S K	0.43	0.32	5.79	26.92	0.0033	0.9851	1.0067	0.7607	1.0211
ClK	4.45	2.95	57.83	6.38	0.0356	0.9376	1.0131	0.8323	1.0267
CaK	16.60	9.73	169.02	3.31	0.1529	0.9516	1.0303	0.9410	1.0286
CuK	24.78	9.16	76.22	4.65	0.2152	0.8161	1.0604	0.9999	1.0642

eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
C K	2.88	7.42	2.65	28.65	0.0047	1.2154	0.8827	0.1349	1.0000
O K	25.50	49.35	96.36	10.09	0.0721	1.1684	0.9071	0.2420	1.0000
AlK	1.27	1.46	12.10	16.47	0.0049	1.0473	0.9546	0.3679	1.0050
P K	0.79	0.79	10.08	18.56	0.0051	1.0303	0.9705	0.6110	1.0136
ClK	13.97	12.20	204.81	4.50	0.1131	1.0012	0.9849	0.7947	1.0181
CaK	5.93	4.58	69.04	5.76	0.0560	1.0173	1.0042	0.8893	1.0433
CuK	49.67	24.20	180.45	3.25	0.4566	0.8763	1.0425	0.9998	1.0493

eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
C K	17.77	33.53	22.52	14.72	0.0342	1.1501	0.9141	0.1672	1.0000
O K	31.00	43.91	127.72	9.87	0.0829	1.1045	0.9376	0.2421	1.0000
AlK	1.33	1.12	16.10	14.51	0.0058	0.9879	0.9826	0.4376	1.0049
S K	0.70	0.50	12.16	15.88	0.0055	0.9910	1.0041	0.7785	1.0205
ClK	10.39	6.64	171.81	4.09	0.0843	0.9433	1.0105	0.8453	1.0173
CaK	2.19	1.24	28.25	8.95	0.0204	0.9577	1.0280	0.9261	1.0468
CuK	36.61	13.06	141.67	3.57	0.3195	0.8220	1.0589	1.0064	1.0547

eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
C K	7.14	16.51	7.42	20.60	0.0122	1.1948	0.8924	0.1434	1.0000
O K	28.06	48.75	118.32	9.82	0.0833	1.1484	0.9165	0.2586	1.0000
AlK	1.49	1.54	15.49	15.20	0.0060	1.0287	0.9634	0.3923	1.0048
ClK	13.30	10.43	203.91	4.28	0.1085	0.9832	0.9930	0.8158	1.0174
CaK	3.49	2.42	42.07	7.67	0.0329	0.9987	1.0118	0.9026	1.0459
CuK	46.52	20.35	172.01	3.30	0.4209	0.8594	1.0478	1.0025	1.0502

Figure 6. EDS results for Cu@CCJ and Cu@AA at inner and outer layers upon the cuticle crystal layer

According to EDS results, copper and lead residues were accumulated on outer layer of eggshells. Regardless of the type of eggshell in SEM images, copper was accumulated in two layers and lead was accumulated in a single layer on the cuticle crystal layer of the eggshell that was determined by EDS. When comparing the copper-accumulating layers, copper amount was found more on the cuticle crystal layer as a percentage. In addition, it was observed that heavy metals could not penetrate into the eggshells unless there was a hole reaching the palisade layer.

CONCLUSION

According to ICP-OES results, amount of loaded metal was higher in calcined eggshells. After the eggshells were calcined, the characteristic bands of CO_3^{2-} at 2516, 1799 and 713 cm^{-1} disappeared, while a new band thought to belong to Ca-O appeared at 447 and 455 cm^{-1} for C-CCJ and C-AA respectively. In Cu@CCJ, Cu@AA, Pb@CCJ and Pb@AA, some physical adhesions were observed on the eggshells as a result of FTIR and SEM analysis. In the spectra of Cu@C-CCJ and Cu@C-AA, there were some shifted bands around 400 cm^{-1}. According to the SEM results, 2-layers adhesion was observed on the smooth surface of the Cu@CCJ and Cu@AA. It was seen that both layers formed according to the EDS spots belong to Cu residue. An irregular adhesion was on the holey surface of the Pb@CCJ and Pb@AA.

Lead uptake is higher in both goose and quail eggshells than copper. That is, when these two species are compared, it is seen that lead uptake is higher.

Regardless of the species, the calcined form facilitates metal uptake. After calcination, the surface of eggshell can mediate to load the metal cations, easily. Calcined form of eggshell may help more porous surface as well as bigger surface area.

When we evaluate the amount of copper and lead over species, the amount of lead uptake shows little difference in almost both species. While copper uptake is less in quail species, it is seen to be higher in goose eggshells. These results reveal two different interpretations. First one, the goose eggshell might be more porous, and second one, the copper atom is smaller in diameter than the lead atom.

In a study, it was concluded that the eggshell pore area decreases with egg size (Chen et al., 2019). If we compare the quail egg size with the goose's one, the inferences are quite meaningful.

REFERENCES

Alaba, P.A., Oladoja, N.A., Sani, Y.M., Ayodele, O.B., Mohammed, I.Y., Olupinla, S.F., Daud, W.M. (2018). Insight into wastewater decontamination using polymeric adsorbents. *Journal of Environmental Chemical Engineering,* 6(2): 1651-1672.

Anjaneyulu, U., Sasikumar, S. (2014). Bioactive nanocrystalline wollastonite synthesized by sol–gel combustion method by using eggshell waste as calcium source. *Bulletin of Materials Science,* 37(2): 207-212.

Awogbemi, O., Inambao, F., Onuh, E.I. (2020). Modification and characterization of chicken eggshell for possible catalytic applications. *Heliyon, 6*(10), e05283; DOI 10.1016/j.heliyon.2020.e05283

Bae, Y.J., Lee, M., Kim, M.S. (2006). One-Photon Mass-Analyzed Threshold Ionization Spectroscopy (MATI) of trans-Dichloroethylene (trans-$C_2H_2Cl_2$): Cation Structure Determination via Franck− Condon Fit. *The Journal of Physical Chemistry A,* 110(27): 8535-8541.

Basaleh, A.A., Al-Malack, M.H., Saleh, T.A. (2019). Metal removal using chemically modified eggshells: preparation, characterization, and statistical analysis. *Desalination and Water Treatment,* 173: 313-330.

Bhatia, S., George-Taylor, M., Merideth, C., Hall Jr., J.H. (1983). Low-temperature infrared spectrum of chlorine nitrate and evidence for the existence of chlorine nitrate (ClOONO). *The Journal of Physica| Chemistry,* 87(7): 1091-1093.

Chen, X., Li, X., He, Z., Hou, Z., xu, G., Yang, N., Zheng, J. (2019). Comparative study of eggshell antibacterial effectivity in precocial and altricial birds using *Escherichia coli*. PLoS ONE 14(7): e0220054; DOI /10.1371/journal.pone.0220054

de Luna, M.D., Flores, E.D., Cenia, M.C., Lu, M.C. (2015). Removal of copper ions from aqueous solution by adlai shell (Coix lacryma-jobi L.) adsorbents. *Bioresource Technology,* 192: 841-844.

Eletta, O., Ajayi, O., Ogunleye, O., Akpan, I. (2016). Adsorption of cyanide from aqueous solution using calcinated eggshells: equilibrium and optimisation studies. *Journal of Environmental Chemical Engineering,* 4: 1367–1375.

Feng, Y., Ashok, B., Madhukar, K., Zhang, J., Zhang, J., Reddy, K.O., Rajulu, A.V. (2014). Preparation and characterization of polypropylene carbonate bio-filler (eggshell powder) composite films. *International Journal of Polymer Analysis and Characterization,* 19(7): 637-647.

Gebru, K. A., Das, C. (2017). Removal of Pb (II) and Cu (II) ions from wastewater using composite electrospun cellulose acetate/titanium oxide (TiO_2) adsorbent. *Journal of Water Process Engineering,* 16: 1-13; DOI 10.1016/j.jwpe.2016.11.008

Hashmi, M. Z., Malik, R. N., Shahbaz, M. (2013). Heavy metals in eggshells of cattle egret (Bubulcus ibis) and little egret (Egretta garzetta) from the Punjab province, Pakistan. *Ecotoxicology and Environmental Safety,* 89: 158-165

Hincke, M.T., Chien, Y.-C., Gerstenfeld, L.C., McKee, M.D. (2008). Colloidal-gold Immunocytochemical Localization of Osteopontin in Avian Eggshell Gland and Eggshell. *Journal of Histochemistry & Cytochemistry, 56*(5): 467–476.

Kaya Kınaytürk, N., Tunalı, B., Türköz Altuğ, D. (2021). Eggshell as a biomaterial can have a sorption capability on its surface: A spectroscopic research. *Royal Society Open Science,* 8(6): 1-14; DOI 10.1098/rsos.210100

Kazemi, A., Abdellahi, M., Khajeh-Sharafabadi, A., Khandan, A., Ozada, N. (2017). Study of in vitrobioactivity and mechanical properties of diopsidenano-bioceramic synthesized by a facile method using eggshell as raw material. *Materials Science and Engineering: C,* 71: 604–610.

King'Ori, A.M. (2011). A review of the uses of poultry eggshells and shell membranes. *International Journal of Poultry Science,* 10(11), 908-912.

Kit, N., Hadibarata, T., Yuniarto, A., Sari, A. (2020). Removal of triphenylmethane dye from aqueous solutions through an adsorption process over waste materials. *Biointerface Res. Appl.,* 10: 5772–5779.

Musonge, P., Harripersadth, C. (2021). The Applicability of Eggshell Waste as a Sustainable Biosorbent Medium in Wastewater Treatment–A Review. *Advances in Wastewater Treatment I,* 91: 171-192.

Naemchan, K., Meejoo, S., Onreabroy, W., Limsuwan, P. (2008). Temperature Effect on Chicken Egg Shell Investigated by XRD, TGA and FTIR. *Advanced Materials Research,* 55-57: 333-336.

Pandit, P.R., Fulekar, M. (2017). Egg shell waste as heterogeneous nanocatalyst for biodieselproduction: Optimized by response surface methodology. *Journal of Environmental Management,* 198: 319-329.

Park, H.J., Jeong, S.W., Yang, J.K., Kim, B.G., Lee, S.M. (2007). Removal of heavy metals using waste eggshell. *Journal of Environmental Sciences,* 19(12): 1436-1441.

Park, M., Hauge, R.H., Margrave, J.L. (1988). Reactions and photochemistry of atomic and diatomic nickel with water at 15 K. *High Temperature Science, 25*(1): 1-15.

Pérez-Huerta, A., Dauphin, Y. (2016). Comparison of the structure, crystallography and composition ofeggshells of the guinea fowl and graylag goose. *Zoology,* 119: 52–63.

Podstawczyk, D., Witek-Krowiak, A., Chojnacka, K., Sadowski, Z. (2014). Biosorption of malachite green by eggshells: mechanism identification and process optimization. *Bioresource Technology,* 160*:* 161-165.

Putra, R.S., Liyanita, A., Arifah, N., Puspitasari, E., Hizam, M.N. (2017). Enhanced electro-catalytic process on the synthesis of FAME using CaO from eggshell. *Energy Procedia,* 105: 289-296.

Queiros, M., Bezerra, M., Feitosa, J. (2017). Composite superabsorbent hydrogel of acrylic copolymer and eggshell: effect of biofiller addition. *Journal of the Brazilian Chemical Society,* 28: 2004–2012.

Reddy, Y., Chary, A., Reddy, S. (2015). DC Ionic Conductivity Study by Two Probe Method on (1-X)$Pb(NO_3)_2$:$XCeO_2$ Composite Solid Electrolyte. *Material Science Research India,* 12(2): 89-94.

Renu, M.A., Singh, K., Upadhyaya, S., Dohare, R.K. (2017). Removal of heavy metals from wastewater using modified agricultural adsorbents. *Materials Today: Proceedings, 4*(9): 10534-10538.

Tangboriboon, N., Suttiprapar, J., Changkhamchom, S., Sirivat, A. (2019). Alternative green preparation of mesoporous calcium hydroxyapatite by chemical reaction of eggshell and phosphoric acid. *International Journal of Applied Ceramic Technology,* 16(5): 1989-1997.

Tatzber, M., Stemmer, M., Spiegel, H., Katzlberger, C., Haberhauer, G., Gerzabek, M. (2007). An alternative method to measure carbonate in soils by FT-IR spectroscopy. *Environmental Chemistry Letters,* 5: 9–12.

Tizo, M.S., Blanco, L.A., Cagas, A.C., Cruz, B.R., Encoy, J.C., Gunting, J.V., Arazo, R.O., Mabayo, V.I. (2018). Efficiency of calcium carbonate from eggshells as an adsorbent for cadmium removal in aqueous solution. *Sustainable Environment Research,* 28(6): 326-332.

Tsai, W., Yang, J., Lai, C., Cheng, Y., Lin, C., Yeh, C. (2006). Characterization and adsorption properties of eggshells and eggshell memrane. *Bioresource Technology,* 97: 488–493.

Veli, S., Alyuz, B. (2007). Adsorption of copper and zinc from aqueous. *Journal of Hazardous Materials,* 149: 226–233.

Waheed, M., Butt, M.S., Shehzad, A., Adzahan, N.M., Shabbir, M.A., Suleria, H.A., Aadil, R.M. (2019). Eggshell calcium: A cheap alternative to expensive supplements. *Trends in Food Science & Technology,* 91: 219-230.

Wallington, T.J., Barnes, I., Becker, K.H., Sehested, J., Nielsen, O.J. (2000). Stability and infrared spectra of mono-, di-, and trichloromethanol. *Chemical Physics Letters,* 322(1-2): 97-102.

Wang, B., Xia, J., Mei, L., Wang, L., Zhang, Q. (2018). Highly efficient and rapid lead (II) scavenging by the natural artemia cyst shell with unique three-dimensional porous structure and strong sorption affinity. *ACS Sustainable Chemistry & Engineering,* 6(1): 1343-1351.

Yang, S.C., Liao, Y., Karthikeyan, K.G., Pan, X.J. (2021). Mesoporous cellulose-chitosan composite hydrogel fabricated via the co-dissolution-regeneration process as biosorbent of heavy metals. *Environmental Pollution,* 286(117324); DOI 10.1016/j.envpol.2021.117324

Yu, W., Hu, J., Yu, Y., Ma, D., Gong, W., Qiu, H., Hu, Z., Gao, H.W. (2021). Facile preparation of sulfonated biochar for highly efficient removal of toxic Pb (II) and Cd (II) from wastewater. *Science of The Total Environment,* 750(141545); DOI 10.1016/j.scitotenv.2020.141545

Yusuff, A. S. (2017). Preparation and characterization of composite anthill-chicken eggshell adsorbent: optimization study on heavy metals adsorption using response surface methodology. *Journal of Environmental Science and Technology,* 10: 120–130.

Zhang, S., Huang, Z., Wang, H., Liu, R., Cheng, C., Guo, Z., Yu, X., He, G., Fud, W. (2021). Separation of wolframite ore by froth flotation using a novel “crab” structure sebacoyl hydroxamic acid collector without $Pb(NO_3)_2$ activation, 389: 96–103.