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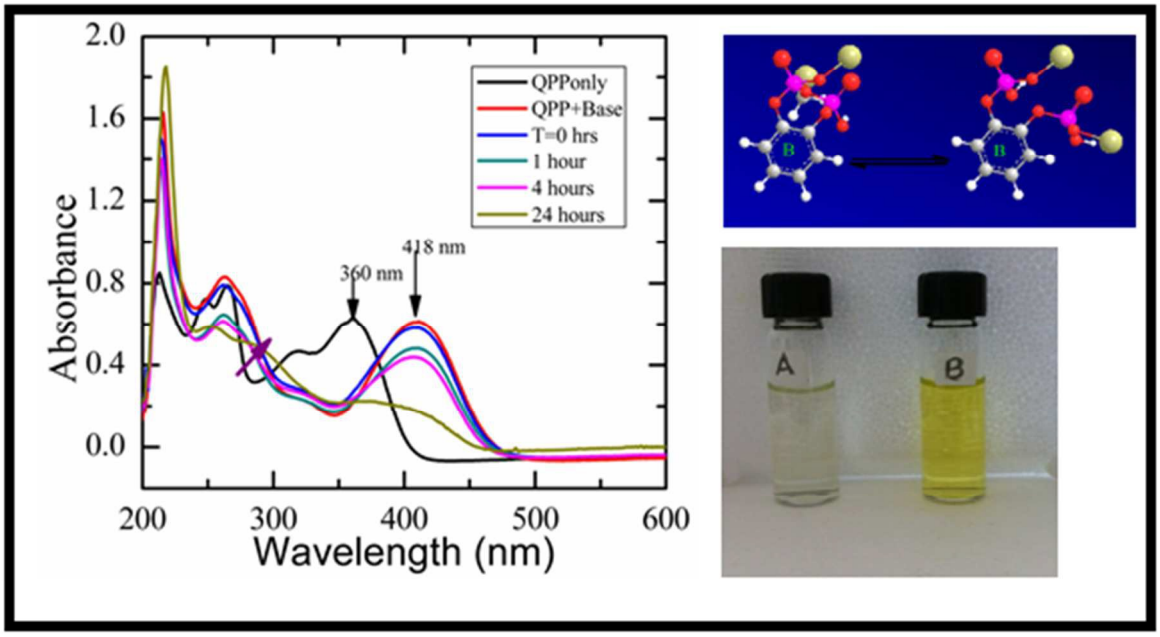

Quantitative removal and in situ detection of lead from water using naturally-derived quercetin pentaphosphate

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Environmental relevance and significance of the studied systems or materials

Green technologies are needed to remove lead from environmental samples in order to achieve the US-EPA maximum contaminant of 0.015 mg/L. This work demonstrates the use of quercetin pentaphosphate (QPP); a naturally-derived quercetin flavonoid to complex and remove lead. The results showed 90.4% and 91.5 % in BRS and BU soil samples respectively and over 91% efficiency indicating effective removal of Pb (II) ions from water samples by QPP *at room temperature. Results are in compliance to the MCL level of 15ppm at ~ 3.82% error margin.*

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ARTICLE

Reactivity, Characterization of Reaction Products and Immobilization of Lead in Water and Sediments using Quercetin Pentaphosphate

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Abstract

Lead is currently ranked the number one heavy metal pollutant with maximum contaminant level (MCL) of 0.015 mg/L. The use of organic-solvent free methods to immobilize lead from the environment is attracting the attention of scientists and environmental engineers. This study reports the application of water soluble Quercetin pentaphosphate (QPP), a derivative of quercetin, for the detection and immobilization of Pb^{2+} from water and soil samples. The techniques employed include UV-visible, fluorescence, atomic absorption; inductively coupled plasma optical emission and Fourier transform infrared (FTIR) techniques. Results indicated the formation of QPP-Pb complex that inhibits fluorescence intensity of the parent molecule. The fluorimetric limit of detection was found to be 3.46 X 10^{-4} M. The QPP-Pb complex exhibited a corresponding stoichiometry with the predominant complex of PX_2. A Scatchard plot of $y = -4*10^6x + 2916.3$ was observed with a negative slope giving an equilibrium constant of $4x10^6$ M^{-1} and $5.4*10^5$ M^{-1} in acidic and alkaline conditions respectively. Results show 90.4 % and 91.5 % lead (II) immobilization from BRS and BU soil samples respectively. On the other hand, 91% lead immobilization efficiency from water sample was achieved at room temperature and is in compliance with MCL level of 15ppm at ~ 3.82% error margin. This approach does not require the use of organic solvents or the disposal of large amounts of sludge. Once complexed with lead, QPP may not release phosphate to cause any secondary pollution.

Key Words: Quercetin Pentaphosphate, lead, immobilization, organic-solvent-free and complexes

1. Introduction

Lead does not exist in nature in its metallic state but can be mined from its ores such as galena (lead sulphide), cerussite (lead carbonate), anglesite (lead sulphate) and wulfenite which form approximately 0.002% of the earth's crust [1]. Its electronic configuration is [Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$ and has 13 isotopes [2]. Most of the lead salts are insoluble in water apart from nitrates and acetates. Pb is currently ranked the number one heavy metal pollutant (and number 2 of all hazardous substances) by the Agency for Toxic Substances and Disease Registry (ATSDR, 2011)[3].

In addition to its abundance and low cost, lead is malleable, resistant to corrosion and has a relatively low melting point. Lead

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† Electronic Supplementary Information (ESI) available Additional information on the synthesis of QPP, Phosphorus NMR , Job's plot, scatchard plots, probable structures of lead -QPP complexation, effect of pH and concentration on QPP-Pb(II) complex formation. This material is available free of charge via Internet. See DOI: 10.1039/x0xx00000x

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is therefore used in different facets of human life, for example automobile industry (storage batteries, petrol/gasoline additives and solders), sheathing of cables, building industries, fishing sinkers and in other applications[1, 4]. Leaded aviation fuel (avtar) is still used in small aviation engines contributing to half of lead pollution in American skies. However it has been phased out of automobile gasoline [5]. Due to the health effects associated with lead exposure; regulatory agencies have banned it's in a number of products leading to significant reduction in the environment [4]. However, due to its non-biodegradability, lead continues to persist in the environment and current exposures are mainly attributed with past product applications [6]. In addition chlorination increases water acidity hence increasing the solubility of lead and other metals in water [1, 7, 8].

In a study carried out at Duke University, researchers found that children living near airports in which lead avgas is present had high levels of lead in their blood [5, 9]. In addition, chronic lead poisoning can lead to decreased bone and muscle growth, among other severe neurological and physiological repercussions [10-12]. Treatment of lead poisoning is mainly achieved through chelating agents [12]. According to the US EPA, the MCL for lead is currently at 0.015 mg/L and with a goal to reducing this level to zero [13]. The removal of lead from waste water has been achieved through a number of techniques including precipitation, solvent extraction, ion exchange, coagulation, floatation and adsorption. Lead adsorption is mostly achieved using natural zeolite, fly ash sub grade, Amberlite IR-120 resin, and granular activated carbon. The first five methods are limited by use of organic solvents, low efficiency and production of large amounts of sludge. It has been established that natural calcite can be used in immobilization of lead and cadmium ions from aqueous solutions [14]. However, the immobilization efficiency needs further improvement. Copper (II) polymethacrylate formed by gamma radiation has been used to immobilize lead (II) ions from wastewater. It is worth noting that lead (II) ions was immobilized in liquid phase via Langmuir-type adsorption mechanism and 60% immobilization efficiency was obtained [15].

We propose the use of a derivative of quercetin, a naturally-occuring flavonoid namely quercetin pentaphosphate (QPP) to immobilize lead; resulting in zero environmental footprint. QPP is easy to synthesize and is very soluble in water (840 mg/mL) [16]. It has been reported in literature that quercetin and other hydroxylated flavonoids can complex with metals therefore making them less available in the environment [17, 18]. However, the only limitation is that flavonoids are less soluble in aqueous solutions hence they require the use of organic solvents. We hereby present the first application of an easy to synthesize water soluble QPP for the immobilization of Pb (II) ions from water samples. The advantage of our method is that it is organic-solvent free and it complexes with lead at ambient conditions.

2. Experimental Section

2.1. Chemicals

All chemicals were of reagent or analytical grade and were used as received without further purification. Lead chloride was from Fisher Scientific, Pittsburgh, PA. Acetonitrile anhydrous (ACN), +99.8%, anhydrous carbon tetrachloride (CCl_4), $\geq$ 99.5%, dibenzyl phosphate, dichloromethane (DCM), palladium 5% on activated carbon, N-N- diisopropylethylamine (DIEA), redistilled, +99.5%, 4-dimethyl aminopyridine (DMAP) were purchased from Sigma-Aldrich, Milwaukee, WI. Anhydrous quercetin was from MP Biomedicals, LLC, Solon, Ohio. Dimethyl sulfoxide-D_6 was from Cambridge Isotope Laboratories, Inc. MA. Ethyl acetate, hexane, methanol, anhydrous sodium sulfate (Na_2SO_4), sodium chloride (NaCl), lead (II) chloride($PbCl_2$) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Fisher Scientific, Pittsburgh, PA. All reagents were prepared using Nanopure water with specific resistivity of 18.0 MΩ cm.

2.2. Instruments

UV/Vis spectra were recorded on an HP 8453 UV-visible diode array spectroscopy at ambient room conditions. The formation of the complexes between QPP and Pb (II) ions was measured by monitoring the changes in UV/Vis spectrum. Fluorescence measurements were carried out on a FLUORAT-02 recording spectrofluorometer from LUMEX Ltd. and processed with Panaroma data analysis software. Perkin Elmer *Spectrum 65* FT-IR spectrometer [Waltham, MA] was used to collect the FT-IR data. The inductively coupled plasma optical emission spectrometry (ICP-OES) model # Optima 7000 from Perkin Elmer was used to analyze the concentration of lead in water samples.

2.3. Synthesis of QPP

The synthesis of QPP (Figure S1A and B) followed the procedure described in literature; however in this case, catalytic hydrogenation was used for the purposes of debenzylation at ambient temperature under atmospheric pressure of hydrogen [16]. The ^{31}P NMR characterization is shown in Figure S1 C and D.

2.4. Determination of pKa of QPP

Manual titration of poly aprotic QPP (0.001 M) was carried out using 0.1 M NaOH.175.5 mg QPP was dissolved in deionized water and made up to the mark of 250 mL volumetric flask to obtain 0.001 M QPP. The resulting solution was titrated with microliter quantities of 0.1 M NaOH delivered using a burette.

2.5. Reaction of QPP and $PbCl_2$

Reaction of QPP and $PbCl_2$ was monitored using both UV-visible and fluorometric techniques. Typical titration experiments were performed by sequential additions of 20 µL of Pb (II) ion solution (1 mM $PbCl_2$ stock solution, to the same 1 mL QPP solution in a quartz cuvette (4.09 µM, from 1 mM stock solution in water). The mixture was equilibrated at 25 °C until no further spectroscopic change was observed. Unless stated otherwise, titration experiments were carried out in both acidic and basic solutions of

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QPP. The fluorescence studies were carried out on emission mode upon excitation at 425 nm and the emission wavelength was scanned from 446 nm to 650 nm.

2.5.1 Effect of concentration on complex formation

In order to determine the effect of concentration on the QPP-Pb complex formation, a series of reaction solutions were investigated at different QPP concentrations ($8.5x10^{-6}$ – $1.25 x10^{-4}$ M) at constant concentration of Pb^{2+} ($7.5x10^{-5}$ M). Change in the absorbance readings were monitored at 421 nm using Uv-Vis Spectroscopy.

2.5.2 Effect of pH on complex formation

The pH of QPP was adjusted to 12.08 using sodium hydroxide solution. The color changed immediately from light yellow to a very deep bright yellow. Thereafter, 40 µL of 278 ppm $PbCl_2$ solution were added and the reaction was monitored using Uv-Vis spectroscopy.

2.6. Job's Method of Continuous Variation

The composition of the Pb (II)-QPP complex was studied with Job's method of continuous variation using equimolar ($2.0x\ 10^{-5}$ mol/L) solutions of Pb^{2+} and QPP at pH 5.2.

2.7 FT-IR Characterization

QPP molecule was characterized before and after complexation with $PbCl_2$ using FTIR. The samples were dissolved in DMSO and then dropped onto an IR Card made of polyethylene (International Crystal Labs. 11 Erie St., Garfield, NJ). The samples were then left in the fume hood for two days to dry and to ensure that all the DMSO solvent had completely evaporated.

2.8 Analysis of Water Samples.

Water samples were spiked with known concentrations of lead chloride salts. The spiked water samples were then treated with QPP and left over night. A yellow precipitate formed which was then filtered out using Whatman filter paper. The filtrate was subsequently analyzed for the presence of Pb^{2+} ions using ICP-OES.

2.9 Environmental Application of QPP for immobilization of lead (II) ions from soil samples

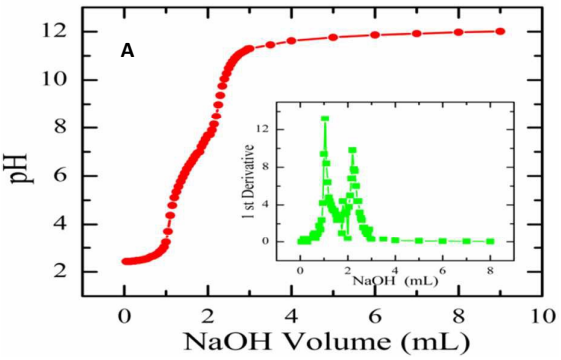
Soil samples were used in addition to water samples to study the efficiency of immobilization of Lead (II) ions by QPP. In this case Binghamton University (BU) soil and Buffalo River Sediment (BRS) [16] were employed. BU soil was ground into fine powder to increase the surface area while BRS samples were used as was obtained. 4.3 g of soil samples were incubated for 24 hours with 3 mL of $7.5 x\ 10^{-5}$ M Pb^{2+}. To the soil samples, 3 mL of $3.57 x10^{-4}$ M QPP and 0.5 M HCl acid were added and the reaction mixture was placed in a water bath at 45 ^{o}C for 120 minutes with vigorous stirring. The control contained soil sample with Pb^{2+} ions without addition of QPP while the blank was soil sample in deionized water without addition of QPP at 45 ^{o}C. Thereafter, the resulting mixtures were then filtered through 0.2 µm acrodisc filter membrane. The efficiency of immobilization of Pb^{2+} was calculated by determining the initial and final absorbance values at 421 nm. The residual Pb^{2+} remaining in the samples were used to determine the % immobilization efficiency.

3. RESULTS AND DISCUSSION

3.1. Determination of pKa

QPP is a derivative of quercetin, a naturally-occurring flavonoid. Previous studies showed that QPP is highly soluble in water (840 mg/mL) and is stable for over 1 year when refrigerated at 4^{o}C [16]. QPP is an example of a polyprotic acid hence it should have more than one equivalence points when titrated with a base. For the QPP (0.001 M) titrations with 0.1 M NaOH, only two equivalence points were observed at ~1.10 mL and ~2.25 mL on all the three trials (Figure 1A). This could correspond to the titration of the protons on the B ring of the QPP. It was not possible to recognize the equivalence points of the other protons on either ring A or C. This is because of the possibility that these occur at too low or too high a pH.

The first derivative plots were used in the accurate determination of these end points. Measurements were further verified by using the Gran plots which locates the end point through the use of data before the end point thus minimizing the effect of the buffer and slow electrode response towards the end point. The titrations were all consistent and yielded a two pK_a values, the first was observed at 2.57 and the second was recorded at 6.23 as shown in Figure 1A inset. These pK_a values were greater than 1 indicating that QPP is a weak acid. This study also further confirmed our previous studies with QPP and Cr (VI) in which HCl had to be incorporated in the reaction in order to improve the kinetics of the overall reaction.



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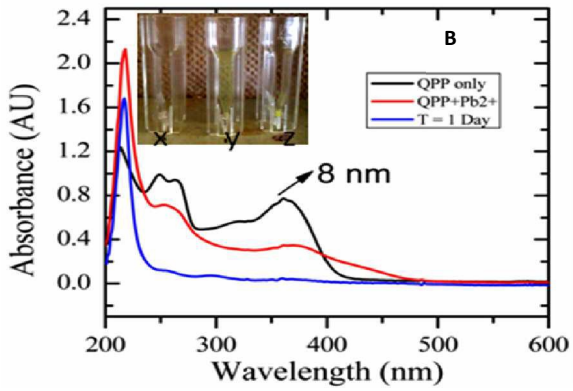


Figure 1. (A) Experimental points in the titration of 0.001 M QPP with 0.1 M NaOH. Inset is the first derivative plot (ΔpH/ΔV) of the titration curve. The end points were taken as maxima in the derivative curve; (B) UV-Vis absorption spectra of QPP in water in the presence and absence of $PbCl_2$ and after 1 day. Spectra of the filtrate from cuvette z (see Figure 1B inset).; inset is a pictogram showing color changes upon addition of Pb^{2+} into QPP solution (X) QPP only (Y) QPP + $PbCl_2$, (Z) QPP + $PbCl_2$ after 1 day.

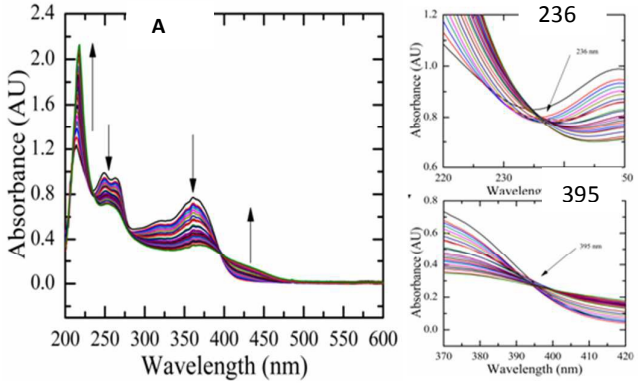
3.2. Reaction of QPP with $PbCl_2$

Similar to quercetin and other flavonoids, QPP possess the ability to complex with metals therefore making them less available in the environment. We have previously carried out an extensive study using QPP and Cr (VI) in which the results showed that QPP can successfully reduce toxic Cr (VI) to its benign form Cr (III) under acidic conditions with the formation of QPP-Cr (III) complex [16]. This implies that, QPP can act as both a reducing agent and a metal chelator. In this study, when a solution of Pb (II) ions was added to QPP, the solution changed color immediately from clear (Figure 1B inset) to yellow indicating that the reaction was instantaneous and a new product was formed. When the resulting solution was left overnight, a yellow precipitate settled at the bottom of the cuvette while the solution color changed back to clear (Figure 1B inset cuvette Z). The corresponding spectrum of cuvettes X, Y and Z are as shown in Figure 1B inset.

QPP is UV active with two characteristic absorption bands at 266 nm (band II) and 360 nm (band I) that are attributed to π⟶π* transitions in the cinnamoyl and benzoyl ring systems [16]. When Pb (II) ions were added, a new band at 440 nm was observed indicating possible Pb-QPP complex formation [16]. The yellow precipitate formed is the QPP-Pb complex because the filtrate did not show the presence of QPP indicating either complete immobilization of QPP from the solution or complete destruction of the cinnamoyl and benzoyl rings on QPP.

Complexation studies of QPP-Pb in water using UV-vis spectroscopy was achieved by varying the molar concentrations of the [$PbCl_2$/QPP]. The spectra obtained are presented in Figure 2A and Figure 2B in acidic and basic media respectively. In Figure 2A, two isosbestic points at 395 nm and 236 nm were observed, when QPP solution was titrated with Pb (II) ions indicating a complex formation in which the respective chromophores were at equilibrium with each other. Both peaks at 266 and 360 nm decreased with subsequent addition of Pb (II) ions indicating either the destruction of both ring A and B on QPP or immobilization of QPP from the aqueous solution.

When the titration of $PbCl_2$ ($1.8e^{-3}$M) was carried out with QPP ($2.5e^{-5}$M) at pH 12.08, an intense peak gradually developed at 240 nm with increase in the concentration of Pb^{2+} within a very wide linear range. However the absorbance peak at 421 nm and the band at 280 nm remained relatively stable with a slight decrease in absorbance values (Figure 2B, inset). The red shift from 360 nm to 418 nm was due to the deprotonation of the QPP molecule in alkaline conditions [16]. A further small red shift of about 5 nm (421 nm) was observed indicating the complexation of the complex formation between deprotonated QPP and Pb^{2+} ions.



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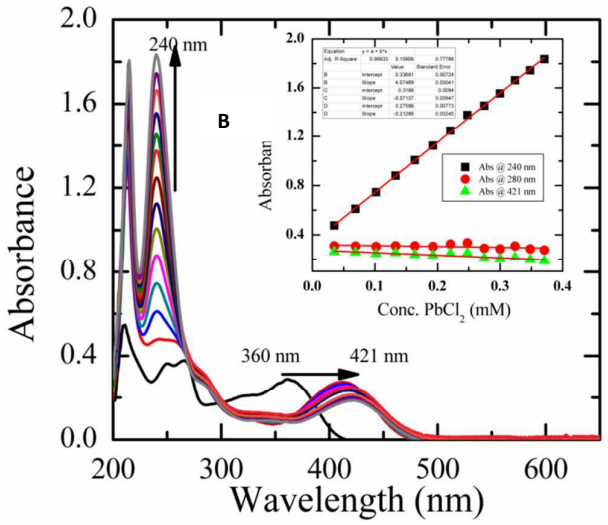

Figure 2. UV-Vis absorption spectra of (A) 6.4x10^{-5}M QPP in water in the presence and absence of 1x10^{-3}M $PbCl_2$. Isosbestic points as observed at pH 3. (B) UV-Vis absorption spectra of QPP in water in the presence and absence of 20µL aliquots $PbCl_2$. Inset is a graph of absorbance versus concentration of $PbCl_2$ at λmax 240 nm, 280 nm and 421 nm. Titration was carried out at pH 12.02.

3.3. Determination of Stability Constant and Stoichiometry

The method of continuous variation was used to determine the identity of the stoichiometry of the predominant complex. The corrected absorbance of each of the equimolar solutions of QPP and Pb (II) was taken and the graph obtained is shown in the supplementary information Figure S2. It is clear that the maximum absorbance was reached at molar fraction of about ≈ 0.7 moles of Pb (II) ions indicating that the corresponding stoichiometry of the predominant complex is PX_2.

The equilibrium constant was obtained using Scatchard plot in which ΔA/[X] was plotted against [X] as shown in Figure S3. Where ΔA is the difference in observed absorbance after each addition of Pb (II) ions and the initial absorbance. Whereas [X] is the final concentration of Pb (II) ions after each addition. As expected, a straight line of y = -4*10^6 x + 2916.3 was observed with a negative slope giving an equilibrium constant of 4 x 10^6 M^{-1} and 5.4*10^5 M^{-1} in acidic (Figure S3A) and alkaline (Figure S3B) conditions respectively. In literature, the stability constants of quercetin (QCR) with various metals had been previously reported. In one particular study by Rajendran et al [19], a 1:1 QCR:Metal ions mole ratio was observed. The metal ions investigated were Pb, Cd and Bi and the stability constant of QCR-Pb in particular as determined by Job's plot was reported to be 1.49 x 10^6 and 9.00 x 10^6 in pH 4.4 and 7.4 respectively which is comparable to the stability constant in our study. In another study by Hajji et al [20], a binding constant of 4 x10^5 M^{-1} for Fe-QCR complex at pH 7.4 was reported. However, a study by Guo et al [21] on the binding constants for QCR with Fe^{2+} was found to be ca 10^6–10^7 M^{-1} (for 1 : 1 complexes) and ca 10^{10}–10^{12} M^{-1} (for 1 : 2 complexes) in phosphate buffer pH 7.2 with the C ring at the 3, 4 position being the preferred chelation site.

A proposed QPP-Pb^{2+} complex structure as drawn by Chemdraw is shown in Figure 3. It should be noted that, there is a possibility that Pb^{2+} could complex with the OH^- groups on rings A and C of QPP; forming a four membered ring structure. However, it is worth noting that this is not a confirmatory structure and future work will focus on determining the exact structure. A study by Cornard reported that Pb^{2+} can chelate with quercetin forming a 2:1, 1:2, and 1:1 species in solution with the ortho-dihydroxy group of B ring (catechol) presenting the highest complexation power toward the Pb(II) ions [22]. In addition, previous bond dissociation studies reported by Guo *et al* showed that the H^1 transfer from the B-ring ring system is easier than from A- ring system [21]. However it is interesting to note that Al on the other hand prefers to coordinate the 3-hydroxy-chromone part of quercetin [23], implying that complexation ratio and patterns will differ from one molecule to another. Furthermore studies on copper-quercetin interaction have employed UV/vis spectroscopy to depict that the interaction of Cu (II) ions with Quercetin yields 2:1 metal: flavonoid ratio [24]. According to literature study, 3-OH and 4-oxo groups have the highest probability of being the first sites for complexation since the 3-OH group has more acidic proton [25]. It was revealed that 3'-4-dihydroxy group in ring B generally binds the second metal ion while the 5-OH group and 4-oxo group have the lowest probability to bind a metal ion. This has been attributed to the fact that 5-OH exhibit lesser proton acidity in addition to the effect of steric hindrance triggered by the first complexation [26].

The other probable structures of lead-QPP complexation Figure S4A is based on the fact that two OH groups on 3'-phosphate and 4'- phosphate groups in the B-ring binds one Pb(II) ion while the OH group 3- phosphate group and 4-oxo bind the other Pb(II) ion. On the other hand, Figure S4B depicts binding at 3'-Phosphate and 4'- phosphate in ring B and at 5-phosphate in ring A and 4-oxo group in ring C.

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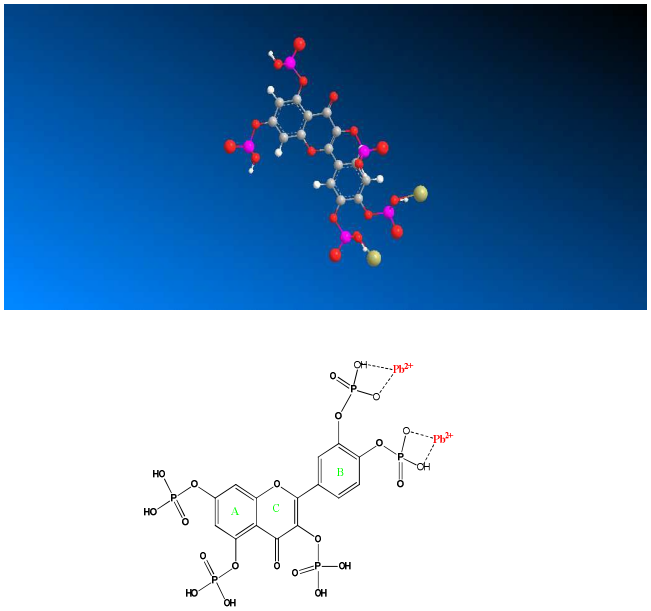

Figure 3. Proposed QPP-Pb (II) complex structure. Red=Oxygen, golden yellow=Pb^{2+}, pink=phosphorus, grey=carbon and white=hydrogen

3.4. Effect of pH on complex formation

When the pH of QPP was adjusted to 12.08 using sodium hydroxide solution, the color of the QPP solution changed immediately from light yellow to a very deep bright yellow color and this was accompanied by a red shift of the wavelength from ~360 nm to ~418 nm (Figure S5). Upon the addition of 40 µL of 278 ppm $PbCl_2$ solution, there was an initial slight decrease in absorption value at the two characteristic absorption bands (262 nm and 418 nm) represented as T=0 min. This could be attributed to the contribution of dilution factor to the total molar concentration of QPP. Thereafter, the absorption bands decreased with time indicating a complete change in both the cinnamoyl and benzoyl ring systems of the QPP molecule. Another interesting observation, as indicated by the purple arrow, was the red shift of the 262 nm band to 285 nm (~23 nm change) after 24 hours of analysis. In addition, the peak at 214 nm increased with time upon the addition of Pb^{2+}. This could be an indication of possible complexation of the QPP with the metal ion.

3.5. Effect of concentration on complex formation

In order to determine the effect of concentration on the QPP-Pb complex formation, a series of reaction solutions were investigated at different QPP concentrations ($8.5x10^{-6}$ – $1.25 x10^{-4}$ M) at constant concentration of Pb^{2+} ($7.5x10^{-5}$ M). Change in the absorbance readings were monitored at 421 nm. Figure S6 shows that the absorbance at 421 nm increased with increase in the concentration of QPP up to $7.5x10^{-5}$ M. Linear range was observed between $8.5x10^{-6}$ and $7.5x10^{-5}$ M.

3.6. Fluorescence studies on QPP-Pb complex

Fluorescence spectrophotometry was also employed to study the QPP-Pb^{2+} complex formed because it is more sensitive than UV-visible spectrometric techniques. The ultra-sensitivity provided by the fluorometric technique is necessary given the relatively low concentrations of lead in the environment. In contrast to many hydroxyflavonols; Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone or QCR does exhibit very little or no fluorescence. However, the QCR-metal complexes have been reported to show highly sensitive molecular fluorescence properties [27, 28]. Similarly, QPP a derivative of QCR absorbs in the UV region with two characteristic maximum at 266 and 362 nm in water at room temperature (Figure 1B), and its fluorescence is extremely weak in acidic conditions compared to the alkaline conditions. Processes that can lead to the reduction of fluorescence intensity can be classified as collisional (O_2, I^-, Cs^+), charge and energy transfer reactions and finally complex formations.

A standard calibration curve of QPP only in alkaline conditions was first developed to eliminate the phenomenon of self-quenching between the molecules of QPP (collisional quenching) (Figure 4A). This enabled the determination of the highest concentration ($1.88 x 10^{-5}$ M) that could be used for complexation studies. QPP emission spectrum was recorded before and after the addition of Pb^{2+}. Upon excitation at 425 nm, QPP exhibited an emission wavelength at 535 nm. However, it was observed that upon complexation with Pb^{2+} in alkaline conditions at excitation wavelength of 425 nm, the fluorescence bands gradually became weaker until a saturation minimum was achieved (Figure 4B & 4C). This is an example of static quenching in which the fluorophore (QPP) forms a non-fluorescent stable complex with Pb^{2+} ions. This implies that the fluorescence will be dependent on the concentration of the quencher as given by the relationship: $I/I_o=1+K_a[Q]$ where K_a is the association constant of the QPP-Pb^{2+} complex, I_o and I are the fluorescence intensities observed in the absence and presence of the quencher [Q] respectively. Therefore a plot of Io/I vs [Q] yielded a linear relationship with R^2=0.992 (Figure 4D).

In addition, it was observed that upon the addition of Pb^{2+} to QPP solution, the emission wavelength was accompanied by a gradual blue shift from ca 530 nm to ca 500 nm due to the QPP-metal complex formed (Figure 4B). At lower concentration of QPP, the complex formed between QPP-Pb^{2+} exhibits a slightly higher sensitive molecular fluorescence activity; this can provide an analytical approach for the detection of heavy metals in environmental water samples. A wide linear range of $6 x 10^{-5}$ – $8 x10^{-4}$ was obtained. The limit of detection (LOD) was calculated as 3 times the standard deviation of the blank and was found to be 0.000346 M.

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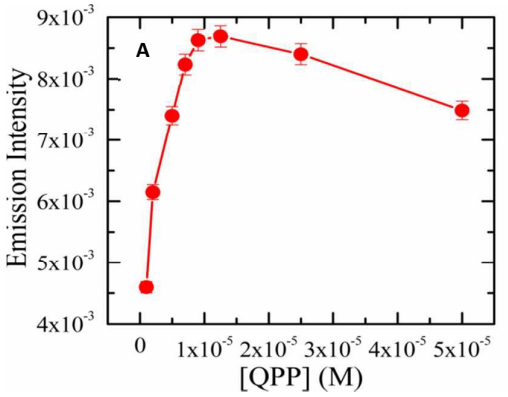

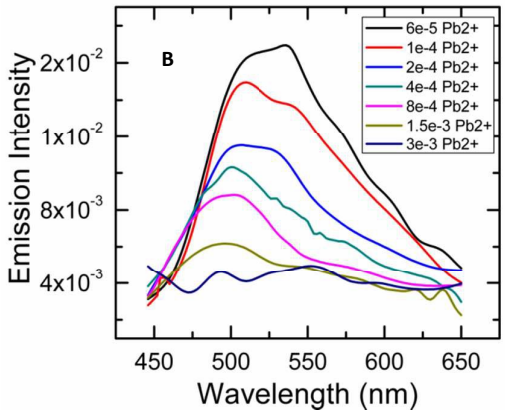

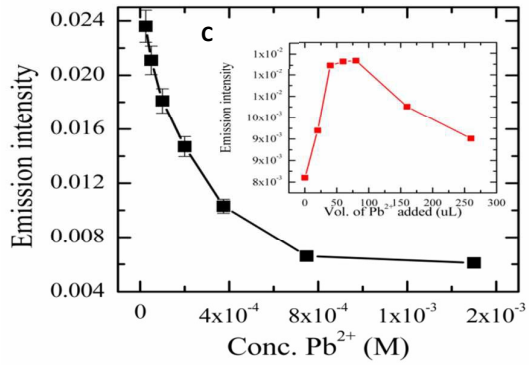

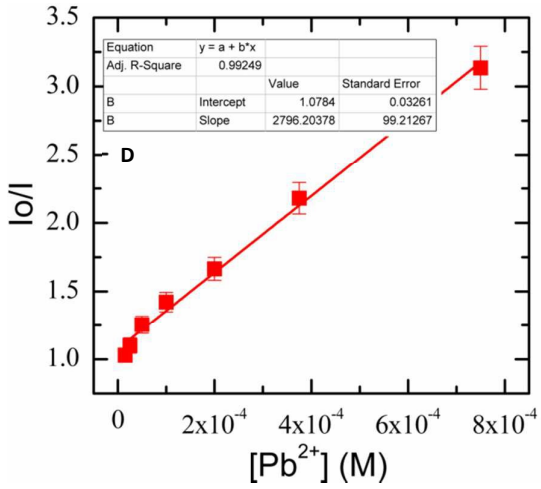

Figure 4. (A) Calibration curve of aqueous QPP solution in alkaline medium featuring dynamic quenching; (B) Graph of fluorescence emission spectrum versus wavelength of QPP-Pb^{2+} in alkaline conditions. (C) Fluorescence intensity change with increasing concentration of Pb^{2+} (measured at $\lambda_{ex}/\lambda_{em}$ = 425/525nm). Inset: fluorescence emission spectrum of QPP at lower concentration of Pb^{2+} (mM). (D) Stern-Volmer plot of QPP in the presence of Pb^{2+}.

3.7. FT-IR Characterization of the QPP and $PbCl_2$ Reaction Product.

In Section 3.2, it was observed that when QPP reacted with lead chloride, a yellow precipitate (QPP-Pb (II) complex) was formed. The yellow precipitate was further characterized using FT-IR technique, and the spectra obtained was compared to those of QCR, QPP and polyethylene (PE) substrates. The results obtained are presented in Figure 5.

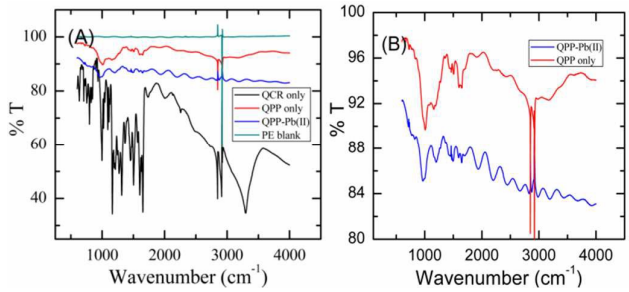

Figure 5. FT-IR Spectra of (A) QCR, PE, QPP and QPP-Pb(II) complex, and (B) QPP and QPP-Pb(II) complex.

The IR spectra of QCR have been reported elsewhere in literature [28]. In this study, the IR spectra of QCR showed the broad band that is due to O-H vibration at ~3300 cm^{-1}, the C=O stretch occurred at 1655 cm^{-1}, while the aromatic C=C bending occurred at 1500 cm^{-1}. The aromatic C-H stretching absorption peak for QCR, QPP and QPP-Pb(II) complex all occurred at 964 nm implying that, complexation did not have any effect on ring A and C system of

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the flavonoid (Figure 5A). The results in Figure 5B showed the absence of O-H stretching band in the QPP-Pb(II) spectrum indicating possible complexation of the hydroxyl groups to Pb(II). However the O-H stretching band in QPP was very broad with a vibration frequency from 3150-3400 cm^{-1} indicating the possible existence of water in the derivatized molecule [28]. Most studies showed that the fourth carbon of C-ring easily binds to metal cations and in addition the oxygen atom of hydroxyl on the 3' and 4' of B-ring also binds to metal cations. We therefore concluded that the most probable QPP-Pb (II) complex structure is as shown in Section 3.3. This is because there was no significant change in the position of the stretching frequency of the C=O bond in the ligand before and after complexation with Pb (II), even though the intensity of the C=O bond in the complex was significantly reduced as compared to that in the free ligand. Also comparing the spectrum of the free ligand to that of the complex, a slight shift was observed at the band occurring at 1200 cm^{-1} further confirming complex formation between QPP and Pb(II) ions. The FT-IR analysis of the reaction product further confirmed presence of complex formation between QPP and Pb (II).

3.9. Efficiency of Immobilization of Pb(II) ions

3.9.1 Immobilization of Pb (II) ions from water by QPP

The efficiency of QPP for the immobilization of Pb (II) ions from water samples was tested using two standard methods AAS and ICP-OES. However, only the results obtained by ICP-OES are presented here given its higher sensitivity as compared to AAS. The detection limit for AAS and ICP-OES [27] is <20 µg/L and <5 µg/L respectively [29, 30]. The % efficiency of this method was calculated using equation 1.

$$\frac{[Pb(II)_i] - [Pb(II)_f]}{[Pb(II)_i]} \, x \, 100 \; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 1$$

Where; $[Pb\ (II)_f]$ = final concentration of Pb (II) after reaction with QPP and $[Pb\ (II)_i]$ = Initial concentration of Pb (II) before reaction with QPP. The results showed over 91% efficiency indicating effective immobilization of Pb (II) ions from water samples by QPP. . QPP is very stable in aqueous solution and our previous work reported stability of over 1 year in aqueous solution [16, 31]. ICP-OES results showed that the metal ion concentrations could be decreased when QPP binds with toxic Pb(II) in water samples and this could lead to inhibition of the lipid peroxidation reactions, and prolong life in case the water sample is consumed by humans or animals either directly or indirectly. The results obtained from ICP-OES are summarized in Table 1.

Table 1. ICP-OES data

Analyte	Net Intensity	Corrected Intensity	Conc. Mg/L	Analysis Time
Blank (HNO_3 2%)	-877.7	-877.7	0.00	15:29:39
Stock Solution (Pb(II))	974897.5	974833.7	209.1	15:38:47
Sample (Pb-analyte)	1798.8	1735.1	0.369	15:43:44

3.9.2 Environmental Application of QPP in immobilization of lead (II) ions from soil samples

The efficiency of immobilization of Pb^{2+} was calculated by determining the initial and final absorbance values at 421 nm as depicted in Figure 6. The residual Pb^{2+} remaining in the samples were used to determine the % immobilization efficiency.

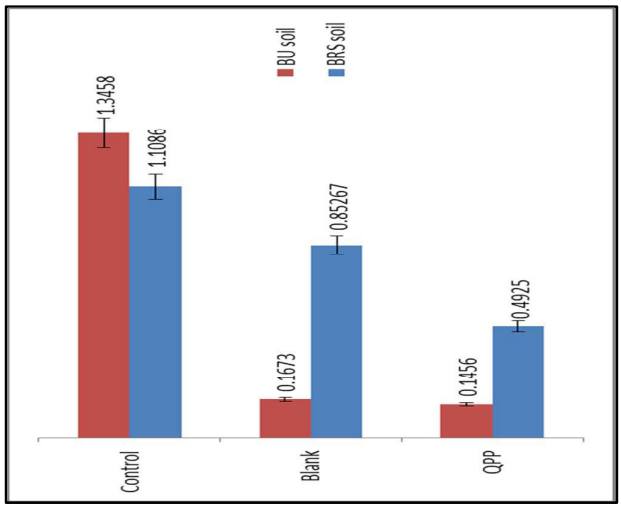

Figure 6. Histograms illustrating control, blank and effect of QPP on soil samples. The control contained soil sample with only $Pb2^{+}$ ions without QPP while the blank was soil sample in deionized water without QPP at 45 °C. The immobilization of Pb^{2+} by interaction of QPP in 4.3 g of soil samples at 45 °C.

QPP showed remarkable ability in remediation of Pb^{2+} from the soil samples by exhibiting immobilization efficiency of 90.4 % and 91.5 % in BRS and BU soil samples respectively. It is worth noting

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that comparatively the % immobilization efficiency was higher in BU soil majorly due to possible reduction agents such as humic acid present in soils.

4.0 Conclusion

The % efficiency of immobilization of Pb (II) ions from water samples was found to be ~ 91% leaving only 15.597 ppb of residual Pb (II) in water which compares to the EPA MCL of 15 ppb [13] at 3.828 % error margin. The % immobilization efficiency of Pb (II) ions were 90.4 % and 91.5 % in BRS and BU soil samples respectively. Two pKa values for QPP were obtained to be 2.57 and 6.23. Fluorometric results showed that QPP is fluorescent in alkaline conditions and that Pb (II) ions diminish the fluorescence intensity of QPP. The limit of detection was found to be 3.46 X 10^{-4} M. The FT-IR analysis of the reaction product further confirmed presence of complex formation between QPP and Pb (II).This study has successfully demonstrated that QPP can successfully be used to complex and immobilize Pb^{2+} in aqueous solutions potentially making toxic metals less available in the environment. This is promising given that QPP is derived from naturally-occurring flavonoids and is highly soluble in water, thereby eliminating the use of organic solvents in environmental remediation. We have conducted studies on stability of QPP in aqueous solutions and we **found** that it was **stable for over a period of 1 year (Reference 16 and 31). Based on these studies we believe that QPP may not release phosphates to cause secondary pollution. Our future work will be focused on at determination of the immobilization efficiency Pb^{2+} in the presence of other metal ions in water.**

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