Morphological and Visible Spectrophotometric Analysis of Copper, Iron, Manganese and Lead Interactions with Extract Chlorophyll

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Abstract: The present paper studies the Interactions of copper, iron, manganese and lead with extract chlorophyll, the most important photosynthetic green pigment, using visible spectrophotometric and morphological analysis. Copper is predominantly replaces the central Mg-atom of the extracted chlorophyll to form a “central complex” of Cu–Chl, whilst this was not proposed in the case of the other three metals (Fe, Mn and Pb), respectively. Instead, the microscopic structures of Chl-HMS products formed in all cases may reflect the possibility for peripheral chelate formation. The latter case may leads to chlorophyll breakdown and the appearance of linear degradation products.

Keywords: Chlorophyll; Heavy Metals; Complexes; Spectrophotometry; Morphology

1. Introduction
Chlorophyll (Chl) ‘the photosynthetic green pigment’ is easily absorb sunlight and starts a chain of electron-transfer steps between carbon dioxide and water through photosynthetic oxidation-reduction reaction. The chemical structure of chlorophyll molecule consists of tetrapyrrole rings with isocyclic cyclopentanone ring fused to the pyrrole ring and the central magnesium (Mg) atom coordinates with four symmetric pyrrole rings as shown in Figure 1. Chlorophyll complexes are made from chlorophyll with several metals as the intention of providing human health with beneficial characterizations. Most complexes are fat soluble and contain different vitamins [1-6]. Chl-Mn complexes are found during the biosynthesis of chlorophyll and may include in degradation of chlorophyll-protein complexes [7, 8]. Cu complexes may affect all kind of photosynthetic activities [9,10]. In general, in vivo [11,12] and in vitro [13] experiments showed the significant impact of heavy metals with the chlorophyll-bound central magnesium atom. Chl-HMS (chlorophyll-heavy metal complex) can be easily formed by heating of the extracted chlorophyll with metal salts in acids or organic solvents. Many researches discussed the extraction and acidification methods of Chl solutions with diluted acids followed by the addition of heavy metal solutions leads to formation of Chl-HMS [14-16]. Chl is considered to be vital in the maintenance of a normal immune system as anti-inflammatory, antioxidant and antibacterial agents. Vitamins A, C and E exist in chlorophyll as helping in neutralize harmful molecules such as free radicals and also the phytoestrogens especially helpful for normalizing estrogen levels. Other Chl complexes play a role in balancing cholesterol levels [17-20]. The pigment complexes are also supposed to help in the treatment of the gastrointestinal syndromes, working as a significant detoxifier and balancing the secretion of digestive fluids. This consequently may relieve severe constipation and decrease odors like bad breath. These complexes can also be beneficial in patients suffering from colitis or ulcers by promoting the production of gastrointestinal mucosal barriers [21-26]. Chl complexes may have a significant future use in the improvement and prevention of cancers. Moreover, the Chl complexes are promoted to improve kidney health by decomposing calcium oxalate grains, to complex heavy metals, and to enhance the releasing of the central magnesium atom [17]. The present research presents the interaction of heavy metals: copper (Cu), iron (Fe), manganese (Mn) and lead (Pb) with the extracted Chlorophyll-bound magnesium using visible spectrophotometry.

2. Experimental
2.1 Extraction of Chlorophylls
Preliminary, natural green pigments were extracted and separated from fresh plant leaves and tissues using standard technique [27]. The relative concentrations of chlorophylls measured in acetone using spectrophotometric equations [28]. Generally, there are two major extracts of photosynthetic pigment were obtained from fragmented leaf tissue; Chlorophyll a (Chl a) and Chlorophyll b (Chl b) with...
minor pigments and carotenoids in which the extracts evaporated and diluted in organic solvent [8]. The concentration of extracted Chl was in the range of 3.9 X 10^{-4} mol/dm^3.

2.2 Preparation of Complexes

After chlorophyll pigment extracted and isolated. The solvent was removed from the Chl-fraction at room temperature. Then 20 ml of individual aqueous solutions of MnSO_4, FeSO_4, CuSO_4 and PbSO_4 were added, respectively. The concentration of metal ions were varied in the range of 0.007– 0.015 mol/dm^3. The time following the formation of the Chl–HMS (t_c) in a period of 3 days. The heavy metals interaction was stopped by dissolving the reaction mixture in cyclohexane. The obtained solutions were dried, centrifuged and allowed to evaporate at room temperature. The samples were dissolved in acetone. The solutions contained not only Chl–HMS complexes, but also unreacted Chlorophyll, as well as may degradation products of chlorophyll formed during the preparation [3].

2.3 Spectrophotometric Analysis

Visible absorption spectra of Chl-HMS complexes in acetone were recorded after different t_c periods using Stat Fax 4500 Spectrophotometer. The same analysis was done jointly with combined metals to observe the combination effects on chlorophylls.

2.4 Morphological Analysis

Morphological structures of free Chl and HMS-Chl products after 3 days of interactions were investigated using Zeiss Primo Star iLED Halogen/LED Microscope 0051.

3. Results and Discussion

3.1. Visible Spectrophotometric Analysis

The visible absorption spectra of the Chl-HMS complexes, after different time periods from the beginning of the complex formation (t_c) are shown in Figs. 2-6, respectively. In all cases, the Chl-content in the blank sample (t_c = 0) is (conc. ~ 10^{-7} mol/dm^3). In general, Chlorophyll has two major absorption bands in the visible spectra (400–800 nm), (Q_y) “red” band and (B) “blue” band [2]. Although the variations were exposed in all systems with the two bands B and Q_y, the interactive effect of the heavy metals (Mn, Fe, Cu and Pb) with extracted chlorophyll was followed using Q_y band, it belongs to chlorophyll only and not to any other pigments [2]. Prior the addition of any heavy metal ions to chlorophyll solutions, a blank test has been done with a view to specify all the probable absorptions to any other factors excluding the HMS–Chl interaction. The blank experiment not shows any perceptible changes in a period of 3 days. It means that changes in visible absorption spectra (as shown in Figs. 2–6) after addition of metal ion solutions of Cu, Fe, Mn and Pb to extract chlorophyll in a period of 3 days should firstly be assigned to the HMS–Chl interactions. Moreover, a hypochromic effect is observed in all cases linked to the maximum absorption Q_y band of Chlorophyll, comparative to the length of the interaction periods. The changes in visible absorption spectra of Cu/Fe-Chl, Fe/Mn-Chl, Mn/Pb-Chl and Cu/Fe/Mn/Pb-Chl solutions at the same metal ratios were detected in a period of 3 days. Clear hypochromic shifts have been observed for Q_y band of Cu-Chl and Cu/Fe/Mn/Pb-Chl solutions (Figs. 2-4, 6), whilst batochromic shift was detected for Mn/Fe/Pb-Chl solutions only relative to t_c (Fig. 5). As a comparison between visible absorption spectra of Cu-Chl solution only and those observed in Cu/Fe/Mn/Pb-Chl solutions. It concludes that in all cases the complex formation of HMS-Chl interactions in a period of 3 days is a strong hypochromic effect of Q_y bands in the corresponding visible absorption spectra at the same time as observed in (Figs. 2-6). The results emphasize that Cu–Chl complex formation is not confused when one or more metals are exist. The effect of Cu only is shown in Fig. 2 (left small plot) related to Cu/Fe and Cu/Fe/Mn/Pb systems in Figs. 2, 6. The absorption spectra behavior shown at Fig. 6 is obviously similar to the one formed by Cu only. On the other hand, the left small plots related to Fe/Mn and Mn/Pb system shows the reverse shift direction. The same, batochromic effect, owing to a supposed Fe–Chl interaction, has been seen for the Fe-Chl solution only (the left small plot). However, this batochromic or “red” shifted absorption may attribute to the presence of chlorophyll derivatives like pheophytins [29]. In Mn-Chl after 3 days of interaction, just a very small hypochromic effect of Q_y band in visible absorption spectra observed. Pb-Chl interaction at the same conditions were examined without any significant change, suggesting that may Mn, Fe and Pb rather form peripheral chelations with chlorophyll than central metal complexes, providing a switched balance between central and chelate Mn–Chl, Fe-Chl and Pb-Chl complexes toward the latter ones [29]. Lastly, when all metals combined at the same ratio (Fig. 6) the gross effect is clearly similar to Cu itself (Fig. 2). The tendency of heavy metal ions to form the “central” Chl–HMS complexes is ordered as: Mg–Chl ~ Pb-Chl ~ Mn-Chl < Fe–Chl < Cu–Chl [3,6]. The results of heavy metals interaction (Mn, Cu, Fe and Pb) with extracted chlorophyll in this research at the same conditions achieved the same order.

Figure 2: Visible absorption spectra of Cu/Fe-Chl complex compared with Cu–Chl complex (left small plot), for different time periods (t_c).
Figure 3: Visible absorption spectra of Fe/Mn-Chl complex compared with Fe–Chl complex (left small plot), for different time periods (t_c).

Figure 4: Visible absorption spectra of Mn/Pb-Chl (left small plot), for different time periods (t_c). Chl complex (left small plot), for different time periods (t_c).

Figure 5: Visible absorption spectra of Fe/Mn/Pb- complex compared with Mn– Chl complex compared with Pb–Chl complex

Figure 6: Visible absorption spectra of Cu/Fe/Mn/Pb-Chl, for different time periods (t_c).

3.2. Morphological Analysis

Figs. 7a-e exhibit the optical microscopic images of free Chl "Mg-Chl" and HMS-Chl products "Cu-Chl, Fe-Chl, Mn-Chl and Pb-Chl", respectively, at room temperature. Microscopic images obtained from free Chl do not manifest any fractal structures (Fig. 7a), whereas those formed from interaction of HMS with Mg-Chl manifest various fractals as shown in Figs 7b-e. In general, the fractals have several branched structures as observed in Mn-Chl and Pb-Chl images (Figs. 7d and 7e) at room temperature. Clear dense fractal structures of Fe-Chl (Fig. 7c) may related to the peripheral chelate formation, whereas in Mn-Chl and Pb-Chl images (Figs. 7d and 7e) at room temperature. The difference between the fractal structures of HMS-Chl images obtained at room temperatures may attributed to the complex formation process, the Chl concentration and the period required for drying. The major formation mechanism which detects the expansion of the fractal structures from chlorophyll based mainly on the concept of molecular diffusion [30, 31]. There are several techniques used to characterize the fractal dimensions of Chl structures as function of concentration such as box counting method [32].
4. Conclusions

This research presents the interactive effects of four heavy metals “Mn, Fe, Cu and Pb” with extract chlorophyll using morphological and visible spectrophotometric analysis. Predominantly, central Cu-Chl complexes formed by the replacement of the central Mg-atom of chlorophyll with Cu-atom, it was not clearly appeared in case of the other three metals. On the other hand, the microscopic morphology of all Chl-HMS products may contemplate the prospect for peripheral chelation. This case may leads to chlorophyll degradation and the formation of stringy products.

References