Research Article

Influence of Nutrient Supplement in the Single Heavy Metal (Pb^{2+}, Cd^{2+}, Cr^{3+}) Uptake and Mineral Nutrients Absorption by Water *Kangkong* (*Ipomoea aquatica forsk*)

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Abstract
The heavy metal contamination in the food crop has posed global concern due to its harmful effects on both humans and animals. Hence, its uptake and bioaccumulation study are in utmost importance. The present study investigated the effects of Cd^{2+}, Pb^{2+}, and Cr^{3+} stress on mineral contents (K^+, Na^+, Ca^{2+}, P, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) in the different parts of water *kangkong* (*Ipomoea aquatica forsk*) by point analysis method. Water *kangkong* cuttings were grown in tap water supplemented with a very small amount of NPK fertilizer and treated with Pb $(NO_3)_2$, Cd $(NO_3)_2 \cdot 4H_2O$, and $K_2Cr_2O_7$ under two soaking solutions – hydroponics solution and tap water solution. Results revealed that Pb^{2+}, Cd^{2+}, and Cr^{3+} alter the mineral nutrient absorption of *kangkong*. Particularly, the approximate concentrations of most mineral ions (K^+, Ca^+, Fe^{2+}, Zn^{2+}, Cu^{2+}, and Mn^{2+}) in the leaves and stems were reduced by Pb^{2+}, Cd^{2+}, and Cr^{3+} exposure, thus making *kangkong* deficient in nutrients when consumed as food. It was also observed that these heavy metals caused a disturbance in K/Ca and K/Na ratios, which could greatly impact water balance. Data also suggest that nutrient optimization may help *kangkong* to develop tolerance to Cd^{2+}, Pb^{2+}, and Cr^{3+} and can be a good strategy to alleviate the accumulation of heavy metals by *kangkong*. The results also indicate that the translocation of ions of Pb, Cd, and Cr from roots to shoots behave differently in the presence of nutrients.

Keywords: *Ipomoea aquatica forsk*, Absorption, Heavy metals, Nutrient uptake, Translocation of ions

1 Introduction

Plants need nutrients for growth, development, and reproduction. Macronutrients, such as nitrogen (N), potassium (P), potassium (K), sulfur (S), calcium (Ca) and magnesium (Mg) as well as micronutrients, such as zinc (Zn), manganese (Mn), nickel (Ni), copper (Cu), iron (Fe), and molybdenum (Mo) serve an essential role in physiological and biochemical processes of plants e.g. photosynthesis, chlorophyll biosynthesis, DNA synthesis, protein modifications, sugar metabolism, and nitrogen fixation [1], [2]. These nutrients are taken up by the roots as ions. It is important that plants must maintain these nutrients in sufficient but not excessive amounts because the optimum concentrations range of most nutrients is narrow [3]. As they grow, plants have developed adaptive mechanisms to take up, translocate, and store these nutrients, as well as to avoid excess accumulation.

Some essential micronutrients, such as Zn^{2+}, Ni^{2+}, and Cu^{2+}, are also considered as heavy metals. These elements are phyto-accumulated or phyto-extracted along with other nutrients and water and absorbed by the roots as ions. The metal ions are then localized in different parts of the plant by the vascular tissues. At elevated concentrations, these metal ions become detrimental to plants causing toxic effects, for example low biomass accumulation, chlorosis, growth and photosynthesis inhibition, water imbalance, altered nutrient assimilation, and ultimately, senescence [2]–[4]. These toxic effects are more prominent with non-essential heavy metals, especially Pb^{2+}, Cd^{2+}, and Cr^{3+}. The movement of these metals across biological membranes is mediated by transport proteins [1]–[5]. On the other hand, it has been reported that plants form reactive oxygen species (ROS) as a response to heavy metal stress, which can damage different macromolecules, such as lipids, proteins, and nucleic acids, thereby affecting mitochondrial respiration and carbohydrate metabolism [1]–[6]. These ROS may also inactivate other metal ions by substituting metalloproteins [4].

Because many plant metal transporters are not very specific, plants can also take up toxic elements including heavy metals. The most vulnerable plants to absorbing heavy metals are aquatic plants such as *kangkong* (variously translated into English as water spinach, or swamp cabbage). *Kangkong* grows well in the Philippines as it favors an environment with a mean temperature of 25 °C [7]. This plant is very resilient and could even survive where wastewater from industry and households' flows [8]. The heavy metal ions absorbed by this plant can be assimilated, which may have toxic effects in humans [9]. This becomes a great concern where most people are not aware that vegetables could also pose a serious health risk because of the normal assumption that all vegetables are safe for consumption. Based on several studies, *kangkong* just like aquatic plants may be contaminated by heavy metals in the environment [7], [8], [10]–[13]. In the Philippines, water *kangkong* is harvested in bodies of water contaminated with heavy metals, posing a danger to human health.

Aside from the risk brought by the toxic effects of heavy metals, food plants grown in contaminated waters have also been reported to be deficient in mineral nutrients. Data in several studies indicate that heavy metals cause a strong Fe, Zn, and Cu deficiency in food plants because of the decreased uptake or immobilization of these nutrients in the roots. A strong disturbance in K and/or Ca levels, which could result in K/Ca ratio changes and could have a great impact on water balance, has also been described [1], [14]. The gathered data confirms that Cd, Cr, and Pb accumulation causes Fe deficiency in plants. It has been proposed that Cd uptake might be inhibited by Mn or other essential nutrients [14].

Some studies investigated the effects of heavy metals on the nutrient balance of food plants, for example rice, spinach, wheat, green peas, corn, and cucumber. For example, Pb causes an imbalance of the minerals K, Ca, Mg, Mn, Zn, Cu, and Fe within the tissues of rice seedlings by physically blocking the transport of these ions to the absorption sites of the roots [15]. In spinach and wheat, it has been reported that Pb^{2+} reduces most mineral ions tested (Na^{+}, K^{+}, Ca^{2+}, P, Mg^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+}) [16]. In peas, it was described that among the 11 nutrient elements (K^{+}, Ca^{2+}, P, N, Mg^{2+}, Na^{+}, S, Cu^{2+}, Mn^{2+}, Zn^{2+}, and Fe^{2+}), only Fe^{2+} and Cu^{2+} concentrations did not change significantly during 12- or 21-days Pb treatment [17]. In maize seedlings, it was reported that the accumulation of Ca^{2+} in roots and its transport to shoots was less affected by Pb than Cd [18]. In cucumber, it was observed that Pb^{2+} and Cd^{2+} prevented the absorption and accumulation of K^{+}, Ca^{2+}, and Fe^{2+} [19].

While considerable information is available on the distribution of heavy metals (Pb^{2+}, Cd^{2+}, and Cr^{3+}) in *kangkong*, little is known about the influence of these toxic metals on the mineral nutrient balance in its different parts. In this regard, the present study investigated the effects of Cd^{2+}, Pb^{2+}, and Cr^{3+} stress, in single systems, on the mineral contents (K^+, Na^+, Ca^{2+}, P, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) of *kangkong* leaves, stems, and roots.

Likewise, different approaches are being utilized to alleviate the toxicity of heavy metals and to reduce their accumulations in food plants. One of the strategies is proper plant nutrition. It was proposed that Pb distribution to the leaves decreased with decreasing nutrient strength due to very low Pb uptake and concentrations in *kangkong* at 100% nutrient strength in the nutrient medium and very high Pb uptake and concentrations at a low nutrient strength. Whereas, the distribution of Cd showed no relation to nutrient levels in the growth medium [11]. There were other similar studies conducted on other dietary crops, such as wheat, where it was recorded edible parts by either fixation of Cd, or by forming insoluble or sparingly soluble phosphates of Cd [20]. As the impact of nutrients on the uptake of heavy metals of *kangkong* is poorly documented and since *kangkong* is an important dietary crop in the country, the present study explored the role of nutrients on the uptake and accumulation of Pb^{2+}, Cd^{2+}, and Cr^{3+} by *kangkong*. Specifically, the effects of heavy metals that plant nutrients minimize Cd accumulation in the stress on mineral contents in the different parts of water *kangkong* (*Ipomoea aquatica forsk*) by point analysis method were studied. The selected mineral nutrients were (K^+, Na^+, Ca^{2+}, P, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}). Except for Na^+, the selected nutrients were initially reported to be essential in *kangkong* in previous studies.

2 Methods

The applied methodology consists of three sections, including, the pre-experiment, the metal ion treatment, and the analysis of the metal ion concentration. In the succeeding sub-sections, the discussions of the procedures from *kangkong* preparations to metal treatments and to the analysis of metal ion concentrations using energy disperse X-ray fluorescence (EDXRF) machine are briefly presented.

Figure 1: *Kangkong* bundles were purchased in a local market in Imus, Cavite, Philippines.

Figure 2: *Kangkong* cuttings before pre-rooting.

2.1 *Pre-experiment*

Pre-experiments were conducted before the experiment proper to discover or confirm the following: a) optimum conditions to grow pre-rooted *kangkong*; and b) suitable soaking time and concentrations of Cd^{2+}, Pb^{2+}, and Cr^{3+}, which the pre-rooted *kangkong* plants could tolerate to enable comparable observations when treated with heavy metals.

The experiments were carried out with pre-rooted *kangkong* (water *kangkong* variety). Pre-rooted *kangkong* was utilized to ensure that any heavy metals absorbed by the *kangkong* plants in their original environment were eliminated prior to the experiment proper. Three weeks before metal treatment experiments, water *kangkong* plants were purchased in a local market in Imus, Cavite, Philippines (Figure 1). All leaves and roots were removed from the stem, and then the stem was cut 6 in from the bottom, preferably having at least two (2) nodes (Figure 2).

The cuttings were thoroughly washed with tap water and placed horizontally, side by side, in layered shoe racks [Figure 3(a)].

(a)

(b)

Figure 3: (a) *Kangkong* cuttings placed horizontally in layered shoe racks during the pre-rooting period of the experiments and (b) NPK (30-10-10) growth fertilizer powder used in the growth solution.

Figure 4: Pre-rooted *kangkong* plants ready for metal treatment experiments.

The *kangkong* cuttings were submerged in a growth solution prepared from tap water and very small amounts of NPK fertilizer powder [Figure 3(b]. Tap water was added to replenish the growth solution and the cuttings were sprayed with tap water every day. After one week, the growth solution was replaced by tap water only and maintained to a level just enough to immerse the cuttings in their horizontal position. The pre-rooting period lasted for three weeks, until the new stems of pre-rooted *kangkong* plants had grown 5–10 cm in length (Figure 4).

2.2 *Metal ion treatment*

The experiments were performed in an improvised greenhouse facility at 25–27 °C, with an abundant source of natural sunlight. There were two sets of heavy metal soaking solutions – hydroponics medium and tap water medium. The metal soaking solutions were prepared by adding metals in fixed concentrations (5 ppm, 20 ppm, and 40 ppm), in the forms of $Pb(NO_3)_2$, $Cd(NO_3)_2{\cdot}4H_2O$, and $K_2Cr_2O_7$ into a pre-determined amount of hydroponics and tap water. There were two sets of control plants: 1) plants grown in hydroponics solution without metals and 2) plants grown in tap water without metals. The hydroponics solution was prepared using the reagents listed in Table 1. All chemicals are reagent grade (supplied by Merck, Philippines) and used without further purification.

Table 1: Chemical reagents of modified Hoagland Solution used in the hydroponics medium during metal treatment

Chemical Name	Formula
Potassium nitrate	KNO_3
Calcium nitrate tetrahydrate	$Ca(NO_3)_2 \cdot 4H_2O$
Ammonium nitrate	NH_4NO_3
Magnesium sulfate	$MgSO_4$
Calcium dihydrogen phosphate	$Ca(H_2PO_4)_2$
Manganese chloride	$MnCl_2$
Ammonium molybdate tetrahydrate	$((NH_4)_6Mo_7O_{24} \cdot 4H_2O$
2-(N-morpholino) ethanesulfonic acid (MES)	$C_6H_{13}NO_4S$
Potassium dichromate	K_2CrO_7

Samples were harvested after the 24th and 48th hours of metal treatment time for the Cd and Cr-treated *kangkong*. Pb-treated *kangkong* plants were not harvested until the 96th hour since these plants were still thriving and healthy. The treatment time was fixed based on preliminary experiments where it was observed, except for the Pb-treated plants. The majority of the Cr- and Cd-treated plants showed necrosis symptoms after 48 h of metal treatment. The choices of concentrations, 5, 20, and 40 ppm, were based on literature and pre-experiment conducted. The harvested samples were thoroughly rinsed twice with distilled water for 2 min, wiped and covered with paper towels, and placed in brown paper bags for analysis.

2.3 *Analysis of metal ion concentration*

The approximate elemental distribution of the selected mineral nutrients in the harvested samples was measured by an X-ray analytical microscope (model: XGT-72000; manufacturer: HORIBA, Ltd.). Three points were analyzed on the leaf, a portion of the stem, and the roots of pre-rooted and metal-treated *kangkong* samples, with the corresponding values, reported as mean. The instrument is capable of doing the hyperspectral mapping analysis of heavy metals in samples using energy disperse X-ray fluorescence

(EDXRF). This is a non-destructive technique in which the freshly harvested samples are directly analyzed without prior preparation. The harvested metal-treated *kangkong* samples were directly scanned without destructive preparation.

The XRF data cube contains both spatial and spectral information. After data acquisition, a user can extract spectra from specified regions within the parts of the *kangkong* making it possible to examine the selected elements for study and perform qualitative/ quantitative spectral analysis from the data set. In the point analysis method, three points were selected in strategic locations in the leaves, stems, and roots of the harvested metal-treated *kangkong* plant. The spectrum at each location was collected automatically by the XGT-7200 Smart Map Software and the approximate elemental distribution was recorded in % mass concentration with respect to all metals present in the *kangkong* sample.

The images in the XRF data cube show the portion of stem, root and *kangkong* leaf viewed with the optical microscope, and spectrum showing the selected mineral nutrients. The mean and the standard deviation of the three selected points for each desired mineral nutrient were then calculated and presented.

3 Results and Discussion

Based on initial readings, harvested samples that were treated with 5 ppm of heavy metals absorbed no heavy metals and very little amounts of the desired mineral nutrients. Also, the majority of the *kangkong* plants treated with 40 ppm of heavy metals showed early signs of necrosis symptoms. Thus, the choice of concentration for analysis, 20 ppm, enabled the present work to observe comparable values of the selected nutrient levels and heavy metal accumulation among the treated *kangkong* plants.

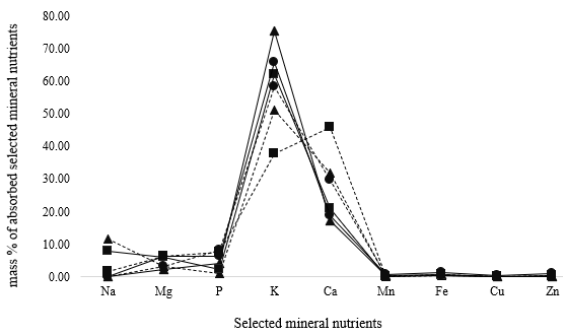

Figure 5: Mass % of absorbed selected mineral nutrients: (▲) control leaf; (■) control stem; and (●) control roots of *kangkong* control plants grown in --- hydroponics medium vs. ― tap water medium.

3.1 *Nutrient supplement altered the absorption of K^+ and Ca^{2+} by kangkong control plants*

Table 2 shows the average values of the selected mineral nutrients in the different parts of the *kangkong* control plants (hydroponic control and tap water control).

Figure 5 shows the mass % of absorbed selected mineral nutrients (K^+, Na^+, Ca^{2+}, P, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) in the leaf, stem, and roots of *kangkong* control plants grown in the hydroponics medium and tap water medium. As evident from Figure 5, K^+ and Ca^{2+} were more sensitive to nutrient supplements than the other mineral nutrients. The control values

Table 2: Mass % of absorbed selected mineral nutrients in the leaves, stems, and roots of *kangkong* control plants

Selected Mineral Nutrients	Leaves		Stems		Roots	
	Tap Water Medium	Hydroponics Medium	Tap Water Medium	Hydroponics Medium	Tap Water Medium	Hydroponics Medium
Na^+	0.00	11.72	7.96	1.60	0.00	0.02
Mg^{2+}	2.12	3.30	6.12	6.30	6.30	3.10
P	4.13	1.11	2.16	7.52	6.45	8.27
K^+	75.44	51.04	61.86	37.80	65.59	58.21
Ca^{2+}	17.24	31.85	21.02	45.80	18.70	29.48
Mn^{2+}	0.27	0.21	0.17	0.15	0.58	0.28
Fe^{2+}	0.56	0.53	0.42	0.61	1.24	0.33
Cu^{2+}	0.14	0.11	0.05	0.04	0.24	0.04
Zn^{2+}	0.09	0.13	0.25	0.17	0.90	0.27

* values are mean of triplicate runs.

indicated that in the absence of additional nutrients, K^+ absorption by *kangkong* was lesser, whereas Ca^{2+} was absorbed more. This observation is more prominent in the leaves and stems of the control plants.

The ions of Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} were not significantly affected by nutrient supplement as their contents have comparable values in the different parts of *kangkong* in both hydroponics and tap water growth media. It was also observed that the roots and leaves of the tap water control plant did not contain Na^+. In the stem, however, control values of Na^+ in the tap water control plant were higher than in the hydroponics control plant.

K^+ content in the plant organs tends to decrease in both control plants according to the following trend: leaves > roots > stems.

3.2 *Nutrient supplement does not change the sink of accumulated Cd^{2+} and Pb^{2+} in kangkong*

In the leaves, the amounts of heavy metals absorbed was in the order of $Cr^{3+} > Pb^{2+} > Cd^{2+}$ under nutrients optimization. Except for Cd^{2+}, which was not found in the leaves, the same trend was also observed for Pb^{2+} and Cr^{3+}under the tap water medium (Figure 6).

In the stems, Pb^{2+} was translocated in the highest amount under the hydroponics medium whereas Cr^{3+} was translocated more under the tap water medium (Figure 7). The ions of Cd^{2+} were not translocated in the stems with or without nutrient supplement. This observation is not in agreement with the accumulation in mesocotyl and coleoptile of maize seedlings under a hydroponics medium, where Cd^{2+} is higher than Pb^{2+} [18].

The data in the experiments indicated that when the root uptake is high, a greater proportion of the metal was retained in the roots. This is probably due to low mobility in the plant because of a strong tendency to bind to the cell walls and phosphate [1], [11].

The present findings indicated that the roots of *kangkong* were the sinks for Cd^{2+} and Pb^{2+} with low amounts being translocated in the shoots and leaves. Similar findings were also indicated for Pb^{2+} and Cd^{2+} [10], [11], [21]–[23] and for Pb^{2+} [24] in studies with *kangkong*. This observation is also similar to a study where the highest concentrations of metals in Pb- or Cd-treated maize seedlings in hydroponic solutions were observed in the roots [25].

By contrast, the absorbed Cr^{3+} was distributed in

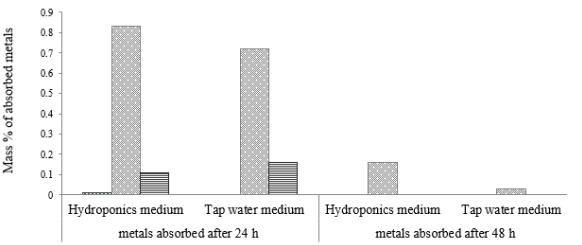

Figure 6: Absorbed (▨) Cd^{2+}, (▩) Cr^{3+}, and (▤) Pb^{2+} by *kangkong* leaves under tap water and hydroponics medium after 24 and 48 h of metal treatment.

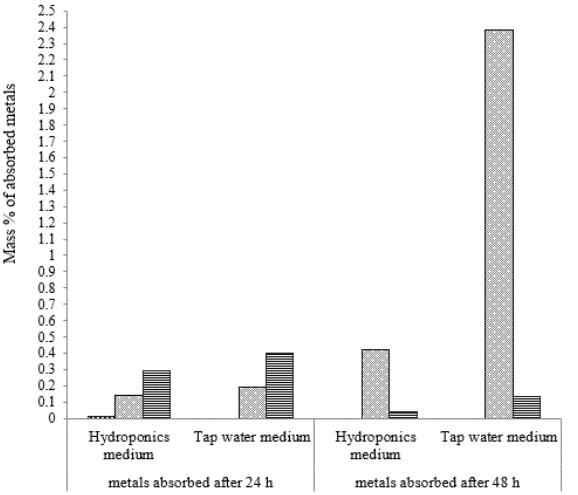

Figure 7: Absorbed (▨) Cd^{2+}, (▩) Cr^{3+}, and (▤) Pb^{2+} by *kangkong* stems under tap water and hydroponics media after 24 and 48 h of metal treatment.

low amounts in roots, stems, and leaves of *kangkong*. Other authors found that the bioaccumulation of Cr^{3+} and Pb^{2+} was greater in the leaves than in the stems of *kangkong* [26], [27].

As shown in Figure 8, the trend of heavy metal accumulation in the roots under nutrient supplement was Pb > Cd > Cr. This is in agreement with the accumulation of Pb^{2+} or Cd^{2+} by maize seedlings roots where Pb^{2+} was accumulated 10-fold higher than that of Cd^{2+} [25]. By contrast, it was reported that Cd^{2+} was accumulated in great amounts in roots in cucumber seedlings but as compared with lead it has the better translocation to cotyledons [19].

In the absence of nutrients, the order of accumulation of heavy metals in *kangkong* roots within 24 h of treatment follows the trend: Cd > Pb > Cr. The results of the experiments indicate that Cd^{2+} is more responsive

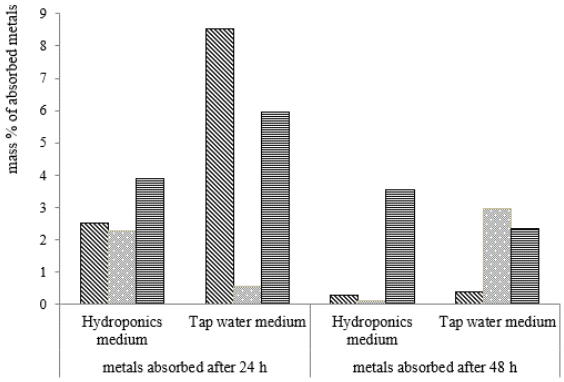

Figure 8: Absorbed (▨) Cd^{2+}, (▩) Cr^{3+}, and (▤)Pb^{2+} by *kangkong* roots under tap water and hydroponics media after 24 and 48 h of metal treatment.

to nutrient supplements than Pb^{2+} and Cr^{3+} as its mass concentration has significantly decreased under the hydroponics medium. The decreased amount Cd^{2+} and Pb^{2+} absorbed after 48 h of metal treatment indicates metal efflux as part of a plant's response and detoxification mechanism [1].

The pattern of distribution of nutrients and heavy metals in *kangkong* can be partly elucidated by the fact that plants assimilate mineral nutrients mainly as cations or anions and the effect on one nutrient may impact the concentrations of the others [1]. This may be due to a higher concentration of plant-available metal species in the medium and less competition with nutrient cations at uptake locations at low nutrient levels [11].

3.3 *Nutrient supplement alleviates Cd^{2+} in kangkong*

It was observed that nutrient supplements decreased the accumulation of Cd^{2+} in the roots by as much as 70.49% after 24 h of metal treatment (Figure 16). This is in line with the observation of other researchers that the application of plant nutrients can efficiently alleviate Cd^{2+} toxicity by decreasing the availability of Cd^{2+} either by fixation of Cd^{2+}, or by forming insoluble or sparingly soluble phosphates of Cd^{2+} [20]. Similarly, optimization of nutrients may decrease the accumulation of Cd^{2+} in plants and alleviate its toxicity by inducing physiological processes and enhancing biochemical reactions. For example, Ca^{2+} alleviates the Cd^{2+} toxicity by reducing its uptake and competing at the transport site, and inducing numerous physiological processes [21]. This experiment also showed a very low amount of Cd^{2+} translocated in the shoots and leaves when nutrients were added to the soaking medium.

On the other hand, the additional nutrients had no clear influence on the distribution of Pb^{2+} and Cr^{3+} to the roots and stems of *kangkong*. While, it was noticed that nutrient supplements reduced the uptake of Pb^{2+} in the roots by 34.5% after 24 h of metal treatment, this trend changed as the soaking time became longer. This is not in agreement with the results of Göthberg *et al.* [11] where it was reported that the lower the nutrient levels in the medium were, the higher Pb concentrations in leaves and stems, and the Cd^{2+} and Pb^{2+} concentrations in roots.

The illustrations in Figures 6 to 8 suggest that mechanisms of translocation of Pb^{2+}, Cd^{2+}, and Cr^{3+} from roots to leaves behave differently in the presence of nutrients.

3.4 *Cd^{2+} Ions: Toxic to kangkong*

3.4.1 Nutrient optimization alleviates Cd^{2+} uptake in the roots of kangkong

It was found that the accumulation of Cd^{2+} in the roots is three-fold without nutrient supplement and that greater uptake occurred during the first 24 h of treatment time (Table 3). The decrease in Cd^{2+} content after 48 h may probably be due to metal efflux as part of a plant's response and detoxification mechanism [1].

Table 3: Cd^{2+} uptake and distribution in *kangkong* leaves, stems, and roots under tap water and hydroponics media after 24 and 48 h of Cd-treatment

Plant Part	Mass % of Cd^{2+} after 24-h Treatment		Mass % of Cd^{2+} after 48-h of Treatment	
	Hydroponics Medium	Tap Water Medium	Hydroponics Medium	Tap Water Medium
Leaves	0.01	0.00	0.00	0.00
Stems	0.01	0.00	0.00	0.00
Roots	2.52	8.54	0.27	0.37

* values are mean of triplicate runs.

As shown in Table 3, the presence of the nutrient solution decreased the accumulation levels of Cd^{2+} in the roots. When nutrients were supplied, i.e., under hydroponics medium, Cd^{2+} uptake decreased in *kangkong* roots by as much as 238.90%, while a very low amount was translocated in the stems and leaves. It was also observed that the accumulation of Cd^{2+} was highest in the roots.

It was proposed that the application of plant nutrients can efficiently alleviate Cd^{2+} toxicity by decreasing its availability either by fixation or by forming insoluble or sparingly soluble phosphates of Cd^{2+} [20]. It was also concluded that the antioxidant properties of Zn^{2+} alleviate Cd-induced oxidative stress in their experiments conducted with *Ceratophyllum demersum* L. grown in a hydroponic medium [22]. By contrast, Göthberg *et al.* [11] observed the distribution of Cd^{2+} in *kangkong* showed no relation to nutrient levels. It was observed that Cd^{2+} was accumulated in high amounts in the roots of other agricultural plants, such as cucumber [19] and maize seedlings [18].

3.4.2 Cd^{2+} induced changes in the absorption of essential metal nutrients in different parts of kangkong

Tables 4 and 5 show the mass % concentration of selected mineral nutrients in different parts of *kangkong* plant after Cd treatment under the tap water medium and the hydroponics medium.

In all parts of the treated plants, Ca^{2+}, Mn^{2+}, Fe^{3+} and Cu^{2+}, and Zn^{2+} were decreased by Cd treatment (Table 6). This observation indicated that Cd^{2+} inhibited the transport of Ca^{2+} ions by channels and transporters [20], [23], [28] and that some plant nutrients, e.g. Ca^{2+}, Fe^{2+}, and Zn^{2+} compete with Cd^{2+} for the same membrane transporters [20], [21].

Table 4: Mass % of selected mineral nutrients after 24 and 48 h of Cd-treatment under tap water medium

Selected Mineral Nutrients	Leaves			Stems			Roots		
	Control	Treatment Time (h)		Control	Treatment Time (h)		Control	Treatment Time (h)	
		24	48		24	48		24	48
Na^{+}	0.00	20.52	10.27	7.96	18.56	1.58	0.00	10.20	0.95
Mg^{2+}	2.12	2.47	6.28	6.12	3.00	4.38	6.3	3.31	5.87
P	4.13	2.45	13.58	2.16	3.00	8.07	6.45	11.16	9.46
K^{+}	75.44	60.25	24.26	61.86	56.20	55.62	65.59	39.82	12.59
Ca^{2+}	17.24	12.64	3.70	21.02	18.51	17.87	18.70	23.57	7.21
Mn^{2+}	0.27	0.61	0.10	0.17	0.11	0.17	0.58	0.18	0.23
Fe^{2+}	0.56	0.69	0.07	0.42	0.34	0.12	1.24	1.61	0.42
Cu^{2+}	0.14	0.24	0.00	0.05	0.11	0.02	0.24	0.48	0.04
Zn^{2+}	0.09	0.13	0.04	0.25	0.16	0.15	0.90	1.12	0.23

* values are mean of triplicate runs.

Table 5: Mass % of absorbed selected mineral nutrients after 24 and 48 h of Cd-treatment under hydroponics medium

Selected Mineral Nutrients	Leaves			Stems			Roots		
	Control	Treatment Time (h)		Control	Treatment Time (h)		Control	Treatment Time (h)	
		24	48		24	48		24	48
Na^{+}	11.72	12.66	2.73	1.60	18.05	1.16	0.02	2.2	12.41
Mg^{2+}	3.30	11.41	5.05	6.30	0.02	6.8	3.10	0.00	2.22
P	1.11	1.78	13.22	7.52	6.19	0.58	8.27	11.09	1.8
K^{+}	51.04	46.59	20.86	37.80	53.56	53.75	58.21	31.31	15.97
Ca^{2+}	31.85	26.2	6.69	45.80	20.73	0.12	29.48	50.05	3.93
Mn^{2+}	0.21	0.51	0.08	0.15	0.44	0.01	0.28	0.38	0.12
Fe^{2+}	0.53	0.55	0.11	0.61	0.73	0.05	0.33	1.13	0.04
Cu^{2+}	0.11	0.1	0.00	0.04	0.12	0.08	0.04	0.27	0.03
Zn^{2+}	0.13	0.19	0.05	0.17	0.16	0.05	0.27	1.02	0.07

* values are mean of triplicate runs.

Table 6: Percent (%) change in the mass concentration of selected metal nutrients in the leaves, stems, and roots of *kangkong* after 24 and 48 h of Cd-treatment under tap water and hydroponics media

Selected Mineral Nutrients	Leaves				Stems				Roots			
	Tap Water Medium		Hydroponics Medium		Tap Water Medium		Hydroponics Medium		Tap Water Medium		Hydroponics Medium	
	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h
Na^+	n.d.	n.d.	7.96	–76.68	133.03	–80.12	1,027.92	–27.50	n.d.	n.d.	11,000.00	61,933.00
Mg^{2+}	16.67	196.38	245.86	53.13	–50.90	–28.34	–99.68	7.88	–47.38	–6.72	–100.00	–28.20
P	–40.76	228.81	60.84	1,094.28	39.26	274.34	–17.69	–92.33	72.88	46.54	34.09	–78.28
K^+	–20.14	–67.85	–8.72	–59.13	–9.14	–10.08	41.67	42.18	–39.29	–80.81	–46.21	–72.56
Ca^{2+}	–26.68	–78.54	–17.75	–79.01	–11.91	–14.97	–54.74	–99.74	26.02	–61.45	69.77	–86.68
Mn^{2+}	124.69	–62.96	141.27	–63.49	–35.29	–1.96	186.96	–95.65	–68.57	–60.00	35.71	–55.95
Fe^{2+}	23.81	–87.50	3.77	–79.25	–20.47	–72.44	19.02	–92.39	29.84	–66.13	238.00	–88.00
Cu^{2+}	69.05	–100.0	–11.76	–100.00	128.57	–57.14	169.23	84.62	98.61	–83.33	583.33	–33.33
Zn^{2+}	48.15	–55.56	48.72	–64.10	–33.78	–37.84	–3.92	–68.63	25.28	–73.98	277.78	–72.84

* values are mean of triplicate runs; n.d. – not detected.

Also, the results in this experiment suggested that Cd^{2+}, even in low amounts (2.52% in root mass concentration), is toxic to *kangkong* by probably altering physiological processes due to decreased contents of K^+ and Ca^{2+} in all parts of the plant while enhancing the uptake of Na^+ in the roots. The mass concentration of Na^+ observed in roots and stems in the hydroponics medium was very significant (Table 6). Addition of Cd^{2+} increased the amount of Na^+ in the roots, and the addition of nutrient supplements increased such uptake. The predicted significant amount of Na^+ accumulated in the roots possibly decreased the concentrations of K^+ as the activities of one influence the other and their transporters and homeostatic mechanisms cannot function independently from each other [1]. The presence of low to moderate amounts of Na^+ in the stem and leaves may have enhanced the toxic effects of Cd^{2+} in *kangkong*.

Visual observation confirmed the toxicity symptoms of Cd-treated plants in both hydroponics and tap water soaking solutions. This might be caused by the significant amount of Na^+ absorbed and the significant loss of K^+ in the roots, stems, and leaves of *kangkong*, as discussed above. It may be concluded that *kangkong* cannot tolerate elevated Na^+ levels and probably expend much energy to remove or exclude it, disturbing regulatory processes and mechanisms. There was also a strong disturbance in K^+ and/or Ca^{2+} levels, which resulted in K^+/Ca^{2+} ratio changes, which could have a great impact on water balance [1], [14], [29], [30]. This may confirm the report that Cd^{2+} permeates through calcium channels, thereby disturbing the plant water status [31].

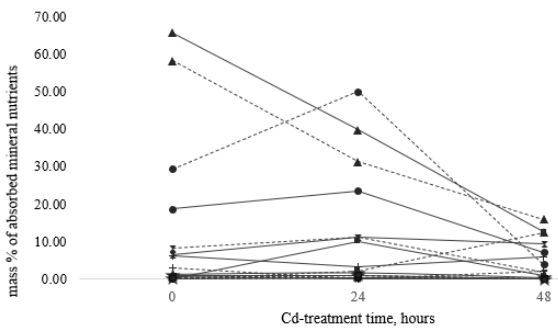

Figure 9: Mass % of absorbed selected mineral nutrients in roots: (■ Na^+, + Mg^{2+}, ⧗ P, ▲ K^+, ● Ca^{2+}, ✖ Mn^{2+}, ♣ Fe^{2+}, ◆ Cu^{2+}, ● Zn^{2+}) after 24 and 48 h of Cd treatment under — tap water and --- hydroponics media.

As shown in Figure 9, 48 h of Cd^{2+} treatment caused a reduction of most mineral nutrients (Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+}) in *kangkong* roots, compared with the controlled values.

Notably, nutrient optimization appeared to alleviate Cd interference in the absorption of Mn^{2+}, K^+, and Cu^{2+}. Specifically, under the hydroponics medium, Mn^{2+} just decreased by –55.95% (as compared with –60.00% under tap water), K^+ by –72.56% (as compared to –80.81% under tap water) and Cu^{2+} by –33.33% (as compared with –83.33% under tap water). Zn^{2+} had no clear response with the nutrient supplement as it was decreased by 73.98 and 72.84%, under the tap water and hydroponics media, respectively. On the other hand, the nutrient supplement further reduced Mg^{2+}, Ca^{2+}, and P by 21.48, 21.87, and 124.82 %,

respectively, more than their decreased values under the tap water medium.

The observed reduction of mineral nutrients in the roots of *kangkong* grown in heavy metals under the hydroponics medium in this study concurs with the findings that Cd^{2+} could interfere with the uptake of various nutrient elements in *kangkong*, probably by its influence in the root membranes, ATP-ases and other carriers as well as by a decrease in roots respiration resulting in a diminished uptake of other elements actively transported into the roots [32]. Similarly, it was also proposed that heavy metals caused a strong Fe^{2+} deficiency by decreasing the uptake or immobilization in the roots of plants [14]. This trend is also similar to the decreases in P, Fe^{2+}, Zn^{2+}, and Cu^{2+} contents observed in bread wheat and all measured element contents of durum wheat grown in a hydroponics medium [33]. A similar reduction in K^+ amount caused by Cd treatment was also found in other agricultural plants, such as cucumber [19], tomatoes [34], and barley [35]. It was also proposed that high doses of Cd^{2+} in nutrient solution caused efflux of K^+ from the roots and that Cd^{2+} had no effect on the absorption and transport of Mg^{2+} [19].

In the stem, Cd^{2+} treatment decreased Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} compared with the controlled values after 48 h (Figure 10).

As seen in Figure 10, Mg^{2+}, K^+, and Cu^{2+} concentrations increased, whereas P decreased under the nutrients supplement. Results showed that the nutrient supplement alleviated the Cd negative effect in the transport of Mg^{2+}, K^+, and Cu^{2+} into the *kangkong* stem. Specifically, Mg^{2+} was increased by 7.88% (as compared to –28.34% under tap water), K^+ also increased by 42.18% (as compared to –10.08% under tap water), and Cu^{2+} by 84.62% (as compared to –57.14% under tap water). On the other hand, the nutrient supplement reduced P by 92.33%, while its value increased by 274.34% under the tap water medium. Similarly, Ca^{2+}, Mn^{2+}, Fe^{2+}, and Zn^{2+} were further reduced with the additional nutrients in the metal soaking medium. Specifically, Ca^{2+} was reduced by –99.74% (as compared to –14.97% under tap water), Mn^{2+} by –95.65% (as compared with –1.96% under tap water), Fe^{2+} by –92.39% (as compared with –72.44% under tap water), and Zn^{2+} by –68.63% (as compared with –37.84% under tap water).

In the leaves, 48 h of Cd treatment decreased K^+, Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} increased Mg^{2+} and P (Figure 11).

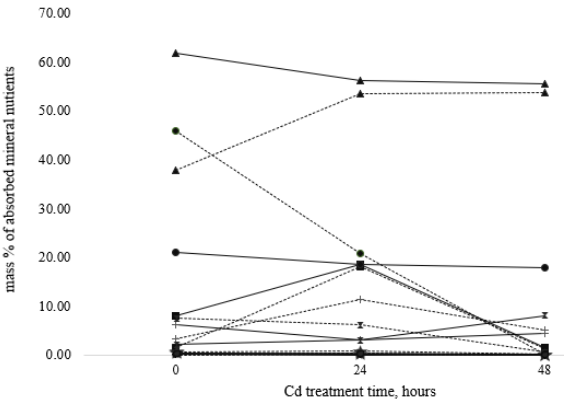

Figure 10: Mass % of absorbed selected mineral nutrients in stems: (■ Na^+,+ Mg^{2+}, ⧗ P, ▲ K^+, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ⬬ Zn^{2+}) after 24 and 48 h of Cd treatment under — tap water and --- hydroponics media.

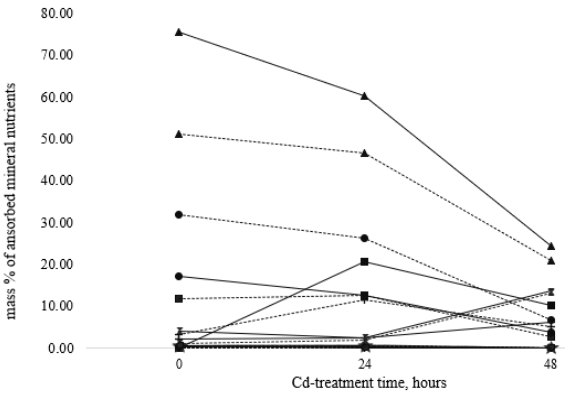

Figure 11: Mass % of absorbed selected mineral nutrients in leaves: (■ Na^+,+ Mg^{2+}, ⧗ P, ▲ K^+, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ⬬ Zn^{2+}) after 24 and 48 h of Cd treatment under — tap water and --- hydroponics media.

As shown in Figure 11, Ca^{2+}, Mn^{2+}, and Cu^{2+} did not show clear responses under nutrient optimization. Results indicated that nutrient supplement alleviated Cd interference to the transport of K^+ and Fe^{2+} up to *kangkong* leaves. Specifically, K^+ was only decreased by –59.13% (compared to –67.85% under tap water), and Fe^{2+} by –79.25% (compared to -87.50% under tap water). On the other hand, nutrient supplements further reduced Zn^{2+} by –64.10% (as compared to –55.56% under tap water), while it increased Mg^{2+} and P by 53.13 and 1,094.28 %, respectively.

3.5 *Pb^{2+} ions: Tolerated by kangkong*

3.5.1 Pb^{2+} uptake was not alleviated by nutrient optimization

While the accumulation of Pb^{2+} was slightly alleviated by the nutrient optimization in the soaking medium after 24 h of metal treatment, the correlation became unclear as a function of treatment time (Table 7).

As presented in Table 7, with or without nutrients amendment, the accumulation of Pb^{2+} was highest in the roots, and a very low amount was translocated in the stems and leaves. It was reported that Pb^{2+} is retained in the roots by binding to ion exchangeable sites on the cell wall and extracellular precipitation in the form of Pb^{2+} carbonate deposited in the cell wall. This results in greater localization of Pb^{2+} ions in the roots than in other parts of the plants [15], [36].

Other studies on the absorption of Pb^{2+} by *kangkong* showed similar results. For example, it was reported that Pb^{2+} concentrations normally decreased from the roots to shoots of *kangkong* harvested in seven locations in Bangkok [10]. Other researchers, after conducting AAS analysis on plant tissues grown in Laguna de Bay, stated that Pb^{2+} concentrations had a decreasing trend from roots to leaves and from bottom to top subsections of the upper 36 cm from the shoot apex [7]. In yet another study, it was implied that Pb^{2+} retention is higher in the root tissues or transport of metal is restricted from roots towards roots [37]. This trend is also similar with other agricultural plants, such as maize seedlings [25], wheat and spinach (*Spinacia oleracea*) seedlings [16] and green peas (*Pisum sativum*) [17].

3.5.2 Pb^{2+} altered the absorption of essential metal nutrients in different parts of kangkong

Tables 8 and 9 show the recorded mass concentrations of selected mineral nutrients after 96 h of Pb treatment of pre-rooted *kangkong* plants under tap water and hydroponics media.

Results indicate that Pb treatments caused changes in the concentrations of most nutrients in the different parts of *kangkong*. Table 10 shows that 48 h of Pb treatment caused a decrease in the concentration of more than half of the selected essential metal nutrients (Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+}) in all parts of *kangkong*.

Table 7: Pb^{2+} uptake and distribution in *kangkong* after 24 and 48 h of Pb-treatment under tap water and hydroponics media

Plant Part	Absorbed Pb^{2+} after 24-h		Absorbed Pb^{2+} after 48-h		Absorbed Pb^{2+} after 96-h	
	Hydroponics Medium	Tap Water Medium	Hydroponics Medium	Tap Water Medium	Hydroponics Medium	Tap Water Medium
Leaves	0.11	0.16	0.00	0.00	0.00	0.29
Stem	0.29	0.40	0.04	0.13	0.09	0.22
Roots	3.89	5.94	3.53	2.35	3.02	1.39

* values are mean of triplicate runs.

Table 8: Mass % of absorbed selected mineral nutrients after 24, 48, and 96 h of Pb-treatment under tap water medium

Selected Mineral Nutrient	Leaves				Stems				Roots			
	Control	Treatment Time (h)			Control	Treatment Time (h)			Control	Treatment Time (h)		
		24	48	96		24	48	96		24	48	96
Na^{+}	0.00	0.00	4.34	5.59	7.96	2.54	4.87	12.48	0.00	0.00	7.86	0.80
Mg^{2+}	2.12	3.49	1.95	2.83	6.12	6.95	2.84	2.86	6.30	1.34	3.29	6.17
P	4.13	1.92	11.59	8.17	2.16	1.83	8.86	5.17	6.45	7.09	4.81	2.80
K^{+}	75.44	60.04	33.93	29.16	61.86	54.21	56.88	40.69	65.59	59.99	31.5	19.13
Ca^{2+}	17.24	33.59	3.75	4.37	21.02	33.14	11.80	8.69	18.70	23.87	3.75	2.93
Mn^{2+}	0.27	0.11	0.05	0.02	0.17	0.01	0.03	0.08	0.58	0.11	0.02	0.05
Fe^{2+}	0.56	0.22	0.09	0.10	0.42	0.88	0.11	0.16	1.24	0.51	0.14	0.10
Cu^{2+}	0.14	0.31	0.09	0.01	0.05	0.00	0.05	0.04	0.24	0.14	0.003	0.66
Zn^{2+}	0.09	0.15	0.05	0.06	0.25	0.01	0.06	0.07	0.90	1.02	0.17	0.13

* values are mean of triplicate runs.

Table 9: Mass % of absorbed selected mineral nutrients after 24, 48, and 96 h of Pb-treatment under hydroponics medium

Selected Mineral Nutrient	Leaves				Stems				Roots			
	Control	Treatment Time (h)			Control	Treatment Time (h)			Control	Treatment Time (h)		
		24	48	96		24	48	96		24	48	96
Na^{+}	11.72	0.00	0.70	3.76	1.60	32.34	0.71	3.04	0.02	12.35	3.79	8.69
Mg^{2+}	3.30	11.56	3.61	5.35	6.30	4.89	2.33	3.49	3.10	17.10	3.20	3.20
P	1.11±	3.40	11.65	7.71	7.52	0.84	9.41	5.13	8.27	5.26	8.47	3.28
K^{+}	51.04	60.04	38.31	28.18	37.80	40.04	63.08	54.37	58.21	34.74	39.23	23.35
Ca^{2+}	31.85	24.02	2.76	8.10	45.80	21.15	8.76	8.17	29.48	24.03	4.03	4.96
Mn^{2+}	0.21	0.07	0.07	0.09	0.15	0.10	0.12	0.08	0.28	0.22	0.02	0.03
Fe^{2+}	0.53	0.30	0.10	0.11	0.61	0.34	0.10	0.11	0.33	1.08	0.14	0.14
Cu^{2+}	0.11	0.11	0.02	0.02	0.04	0.00	0.03	0.04	0.04	0.64	0.02	0.66
Zn^{2+}	0.13	0.39	0.03	0.02	0.17	0.00	0.05	0.07	0.27	0.69	0.14	0.14

* values are mean of triplicate runs.

Table 10: Percent (%) change in the mass concentration of selected metal nutrients in *kangkong* leaves, stems, and roots after 24 and 48 h of Pb-treatment under tap water and hydroponics media

Selected Mineral Nutrients	Leaves				Stems				Roots			
	Tap Water Medium		Hydroponics Medium		Tap Water Medium		Hydroponics Medium		Tap Water Medium		Hydroponics Medium	
	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h
Na^{+}	n.d.	n.d.	–100.00	–94.00	–68.15	–38.80	1,921.46	–55.83	n.d.	n.d.	61,650.00	18,833.30
Mg^{2+}	64.78	–8.02	250.20	9.49	13.68	–53.62	–22.47	–63.04	–78.77	–47.75	452.21	67.66
P	–53.51	180.63	207.53	952.71	–15.15	310.82	–88.78	25.14	9.81	–25.41	–36.42	2.34
K^{+}	–20.41	–55.02	17.63	–24.93	–12.36	–8.05	5.92	66.85	–8.54	–51.86	–40.33	–40.33
Ca^{2+}	94.84	–78.23	–24.59	–91.34	57.70	–43.85	–53.82	–80.87	27.61	–79.93	–18.50	–86.33
Mn^{2+}	–59.26	–81.48	–66.67	–65.08	–92.16	–80.39	–32.61	–21.74	–81.71	–96.57	–21.43	–92.86
Fe^{2+}	–60.12	–83.93	–43.40	–81.76	108.66	–73.23	–45.11	–84.24	–58.60	–88.71	224.00	–58.00
Cu^{2+}	119.05	–100.00	–2.94	–82.35	–100.00	14.29	–100.00	–38.46	–43.06	–98.61	1500.0	–58.33
Zn^{2+}	70.37	–40.74	197.44	–76.92	–97.30	–77.03	–100.00	–68.63	14.13	–81.04	156.79	–46.91

* values are mean of triplicate runs; n.d. – not detected.

In all parts of the treated *kangkong* plants, Ca^{2+} was decreased by Pb treatment. This observation indicates that Pb inhibited the transport of Ca^{2+} ions by channels and transporters [23], [28]. A study on maize seedlings produced an opposite result. wIt was reported that Pb^{2+} accumulation increased the uptake of Ca^{2+}, where the elements were measured in 15 mm-long apical root segments as well as in whole mesocotyls and coleoptiles of maize seedlings after 24-h of incubation in hydroponic solutions.

A similar reduction in most mineral nutrients caused by Pb treatment was also observed in other agricultural crops including wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings grown under hydroponic conditions [16]. It was also reported that only Fe^{2+} and Cu^{2+} concentrations did not change significantly out of 11 nutrient elements assessed in peas (*Pisum sativum*) exposed to 0.5–9.4 mmol lead acetate kg^{-1} dried wt soil for 12 and 21 days in potted soil cultures [17].

On the other hand, the effect of lead applications differs in water hyacinth leaves, where Pb did not decrease the nutrient contents except copper content [38]. It was reported that water hyacinths tolerate Pb toxicity via an increased level of antioxidative enzymes [39]. Likewise, it was also determined that water hyacinth displayed tolerance to increasing doses of lead. It was found that lead applications did not affect the nutrition of water hyacinth. This plant tolerates heavy metal stress and is recognized as a hyperaccumulator plant [38].

As shown in Figure 12, 48 h of Pb treatment decreased the root concentrations of K^{+}, Ca^{2+}, Mn^{2+},

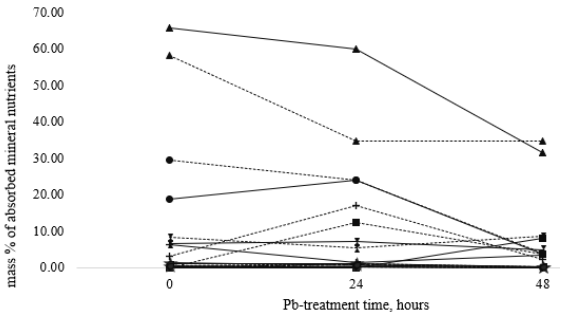

Figure 12: Mass % of absorbed selected mineral nutrients (■ Na^{+},+ Mg^{2+}, ⧗ P, ▲ K^{+}, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ⬬ Zn^{2+}) in *kangkong* roots after 24 and 48 h of Pb treatment under — tap water and --- hydroponics media.

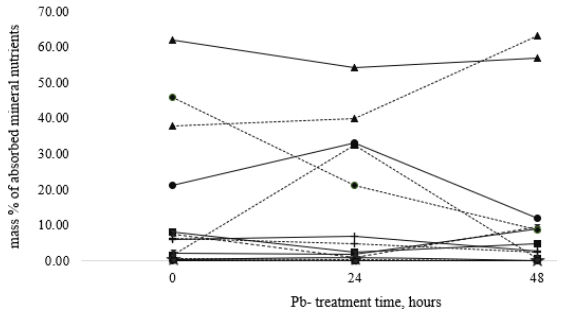

Figure 13: Mass % of absorbed selected mineral nutrients (■ Na^{+},+ Mg^{2+}, ⧗ P, ▲ K^{+}, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ⬬ Zn^{2+}) in *kangkong* stem after 24 and 48 h of Pb treatment under — tap water and --- hydroponics media.

Fe^{2+}, Cu^{2+}, and Zn^{2+}compared with the controlled values. Notably, nutrient optimization increased Mg^{2+} and P uptake in *kangkong* roots by 67.66 and 2.34%, respectively. Also, results indicate that nutrient supplements lessen Pb restriction in the absorption of K^{+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+}. Specifically, K^{+} uptake was only decreased by –40.33% (as compared with –51.86% under tap water), Mn^{2+} by –92.86% (as compared with –96.57% under the tap water), Fe^{2+} by –58.00% (as compared to –88.71% under the tap water), Cu^{2+} by –58.33% (as compared by –98.61% under tap water) and Zn^{2+} by –46.91% (as compared with –81.04% under the tap water). On the other hand, the nutrient supplement further reduced Ca^{2+} by –86.33% as compared with a –79.93 % decrease under the tap water medium.

The observation in this study agrees with the previous report that Pb alters the amounts of K^{+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{2+} within the plant tissues by physically blocking the transport of these ions to the absorption sites of the roots [15]. Similarly, it was also proposed that Pb^{2+} caused leakage of K^{+} ions from root cells in corn seedlings grown in the hydroponics solution [18]. An older study with cucumber showed that Pb^{2+} prevented the absorption and accumulation of K^{+}, Ca^{2+}, and Fe^{2+} and that the high doses of Pb^{2+} in the nutrient solution caused the efflux of K^{+} from the roots [19].

In the stem, Pb treatment decreased Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, and Zn^{2+} (Figure 13). Results show that the nutrient supplement appears to alleviate Pb interference to the transport of K^{+}, Mn^{2+}, and Zn^{2+} to the stem of *kangkong*. Specifically, K^{+} increased by 66.85% (as compared to –8.05% under tap water), while Mn^{2+} was only decreased by –21.74% (as compared with –80.39% under tap water) and Zn^{2+} by –68.63% (as compared with –77.03% under tap water). On the other hand, the nutrient supplement further reduced Mg^{2+} transport by –63.04% (as compared with –53.62% under tap water), Ca^{2+} by –80.87% (as compared with –43.85% under tap water), Fe^{2+} by –84.24% (as compared with –73.23% under tap water). Also, Pb treatment decreased Cu^{2+} by –38.46% under the hydroponics medium, whereas under the tap water medium, it increased by 14.29%.

As shown in Figure 14, Pb decreased the concentrations of K^{+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} in the leaves of *kangkong*. Nutrient optimization alleviates Pb interference to the transport of K^{+}, Mn^{2+}, and Cu^{2+}. In particular, K^{+} was only decreased by –24.93% (as compared with –55.02% under tap water), Mn^{2+} by –65.08% (as compared with –81.48% under tap water), Cu^{2+} by –82.35% (as compared with –100% under tap water). The ions of Fe^{2+} has no clear response with the nutrient supplement as it decreased by 83.93% under the tap water medium, while it was reduced by 81.76% under the hydroponics medium. On the other hand, the nutrient supplement further reduced Ca^{2+} by 13.11% more and Zn by 36.18% more than their decreased value under the tap water medium. Pb treatment enhanced the transport of P in *kangkong* leaves as it increased by 772.08% under the hydroponics medium as compared with its increase under the tap water medium, which was only 180.63%.

As the results show, the K^{+} and Ca^{2+} concentrations decreased in the roots and stems after the 48-hour Pb

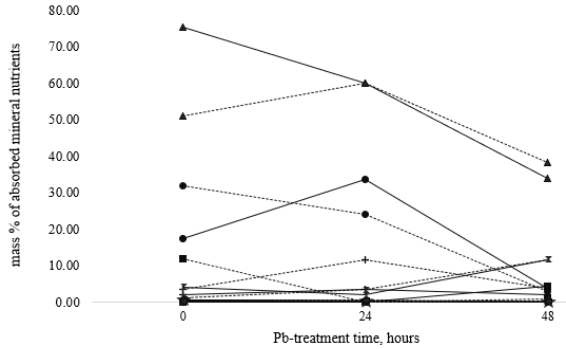

Figure 14: Mass % of absorbed selected mineral nutrients (■ Na^+,+ Mg^{2+}, ⧗ P, ▲ K^+, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ⬬ Zn^{2+}) in *kangkong* leaves after 24 and 48 h of Pb treatment under — tap water and --- hydroponics media.

treatment period. The K^+ concentration values decreased in the roots and stems during the treatment period under the hydroponics medium whereas its values increased in the stems. The addition of nutrients decreased the mass concentration of Ca^{2+} in all parts of the plant. As indicated by the results, the nutrient supplement enhanced the root absorption of Na^+ in Pb-treated *kangkong*. The highest mass concentration of Na^+ was recorded in the roots after 24 h of treatment, as much as 61,650% increase, but eventually decreased to 18,833.3% after 48 h.

While Pb reduced most of the essential nutrients, it was interesting to note that treated plants were healthy and appeared to tolerate 20 ppm of Pb until all of the soaking solutions was consumed, which took 22 days. The treated plants did not show any signs of toxicity symptoms. The results of the experiments show that the concentration of the absorbed Pb in the roots decreased over time, probably due to Pb efflux and other detoxification mechanisms of *kangkong* in response to metal exposure [1]. Also, it appears that *kangkong* was able to mitigate the movement of Na^+ from the roots into the shoots and leaves.

This experiment suggests that *kangkong* can tolerate 20 ppm of Pb in a growth medium. As evidenced by the acquired data, this plant is likely to survive and grow in Pb contaminated waters even in the absence of nutrient supplements. Moreover, it is likely that this plant could develop efficient mechanisms to tolerate Pb toxicity. This is in contrast with the observation by Chanu and Gupta in 2016 where it was reported that *kangkong* started to show toxicity symptoms after 12 days of exposure in 20 ppm of Pb [37].

3.6 *Cr^{3+} Ions: Toxic to Kangkong*

3.6.1 Cr^{3+} uptake was not alleviated by nutrient optimization

Table 11 shows that absorption of Cr^{3+} by *kangkong* has no clear response upon nutrient optimization in the growth medium. Other studies reported that Si alleviates the Cr^{3+} toxicity in rice plants by inhibiting the uptake and transport of Cr^{3+} and enhancing a defense mechanism against oxidative stress caused by Cr^{3+} toxicity [40].

It was also reported that the use of nutrient supplements, particularly nitrate, favors both bioaccumulation and the transport of Cr^{3+} from the roots to the foliage in maize plants [41]. As observed in the present experiments, *kangkong* treated with Cr showed toxicity symptoms at 20 ppm with or without nutrient optimization as the soaking time became longer.

It was observed that the absorbed Cr^{3+} was distributed in low amounts in the roots, stems, and leaves of *kangkong*. It was also reported that *kangkong* has uniform absorption characteristics showing over 75% removal of added Cr^{3+} in the growth medium optimized with nutrients [42]. Also, it was found that *kangkong* tends to accumulate Cr^{3+} in roots and shoots, and the accumulation in the roots was enhanced by the addition of EDTA [43]. By contrast, Jha *et al.* [24] in 2016 also found no substantial accumulation of Cr^{3+} in *kangkong* grown naturally in India.

Table 11: Cr^{3+} uptake and distribution in the leaves, stem, and roots of *kangkong* after 24 and 48 h of Cr-treatment

Plant Part	Absorbed Cr^{3+} after 24-h		Absorbed Cr^{3+} after 48-h	
	Hydroponics Medium	Tap Water Medium	Hydroponics Medium	Tap Water Medium
Leaves	0.83	0.72	0.16	0.03
Stem	0.14	0.19	0.42	2.38
Roots	2.26	0.54	0.11	2.96

* values are mean of triplicate runs.

3.6.2 Cr^{3+} altered the mineral nutrient absorption of kangkong

Tables 12 and 13 show the recorded mass concentration of the selected mineral nutrients in *kangkong* after 48 h of Cr treatment. As shown in Table 14, Cr treatment caused changes in the concentrations of most nutrients in the different parts of *kangkong*.

Table 12: Mass % of absorbed selected mineral nutrients after 24 and 48 h of Cr-treatment under tap water medium

Selected Mineral Nutrients	Leaves			Stems			Roots		
	Control	Treatment Time (h)		Control	Treatment Time (h)		Control	Treatment Time (h)	
		24	48		24	48		24	48
Na^{+}	0.00	7.30	2.70	7.96	2.36	2.21	0.00	18.53	0.12
Mg^{2+}	2.12	2.09	4.18	6.12	0.31	3.25	6.30	2.84	17.10
P	4.13	9.06	18.68	2.16	8.69	9.67	6.45	8.57	6.20
K^{+}	75.44	65.55	27.49	61.86	69.56	58.17	65.59	59.07	23.25
Ca^{2+}	17.24	13.99	2.60	21.02	18.03	10.36	18.70	9.92	6.09
Mn^{2+}	0.27	0.26	0.04	0.17	0.19	0.097	0.58	0.06	0.05
Fe^{2+}	0.56	0.78	0.05	0.42	0.57	0.183	1.24	0.30	0.23
Cu^{2+}	0.14	0.16	0.003	0.05	0.03	0.003	0.24	0.03	0.02
Zn^{2+}	0.09	0.10	0.037	0.25	0.07	0.10	0.90	0.14	0.24

* values are mean of triplicate runs.

Table 13: Mass % of absorbed selected mineral nutrients after 24 and 48 h of Cr-treatment under hydroponics medium

Selected Mineral Nutrients	Leaves			Stems			Roots		
	Control	Treatment Time (h)		Control	Treatment Time (h)		Control	Treatment Time (h)	
		24	48		24	48		24	48
Na^{+}	11.72	0.00	7.27	1.60	17.90	5.82	0.02	0.98	1.47
Mg^{2+}	3.30	0.63	4.11	6.30	4.76	2.47	3.10	0.36	17.10
P	1.11	15.54	15.68	7.52	13.11	10.38	8.27	11.07	12.29
K^{+}	51.04	46.79	20.40	37.80	43.91	46.20	58.21	48.31	34.07
Ca^{2+}	31.85	35.24	6.32	45.80	19.68	18.29	29.48	32.85	11.66
Mn^{2+}	0.21	0.44	0.06	0.15	0.09	0.06	0.28	0.76	0.07
Fe^{2+}	0.53	0.33	0.19	0.61	0.31	0.19	0.33	1.14	0.59
Cu^{2+}	0.11	0.09	0.01	0.04	0.03	0.01	0.04	0.65	0.14
Zn^{2+}	0.13	0.11	0.02	0.17	0.07	0.02	0.27	1.60	0.03

* values are mean of triplicate runs.

Table 14: Percent (%) change in the mass concentration of selected metal nutrients in *kangkong* leaves, stems, and roots after 24 and 48 h of Cr-treatment under tap water and hydroponics media

Selected Mineral Nutrients	Leaves				Stems				Roots			
	Tap Water Medium		Hydroponics Medium		Tap Water Medium		Hydroponics Medium		Tap Water Medium		Hydroponics Medium	
	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h	24-h	48-h
Na^{+}	n.d.	n.d.	–100.00	–37.97	–70.36	–72.29	1,018.75	263.75	n.d.	n.d.	4,800.00	7,250.00
Mg^{2+}	–1.42	97.17	–80.91	24.55	–94.99	–46.81	–24.48	–60.81	–54.90	171.57	–88.37	452.21
P	119.37	352.30	1,304.22	1,316.87	303.09	348.38	74.41	38.09	32.75	–3.98	33.80	48.55
K^{+}	–13.11	–63.56	–8.32	–60.03	12.45	–5.96	16.15	22.21	–9.94	–64.55	–17.01	–41.47
Ca^{2+}	–18.85	–84.92	10.63	–80.16	–14.20	–50.69	–57.03	–60.07	–46.96	–67.44	11.42	–60.45
Mn^{2+}	–3.70	–85.19	109.52	–66.67	9.80	–43.14	–41.30	–60.87	–89.71	–91.43	171.43	–75.00
Fe^{2+}	39.29	–91.07	–37.74	–79.25	35.43	–56.69	–49.46	–69.02	–75.54	–80.91	242.00	77.00
Cu^{2+}	14.29	–97.86	–20.59	–91.18	–35.71	–92.86	–30.77	–70.00	–88.89	–91.67	1,525.00	250.00
Zn^{2+}	11.11	–55.56	–15.38	–84.62	–72.97	–59.46	–57.06	–88.24	–84.01	–73.61	492.59	–88.89

* values are mean of triplicate runs; n.d. – not detected.

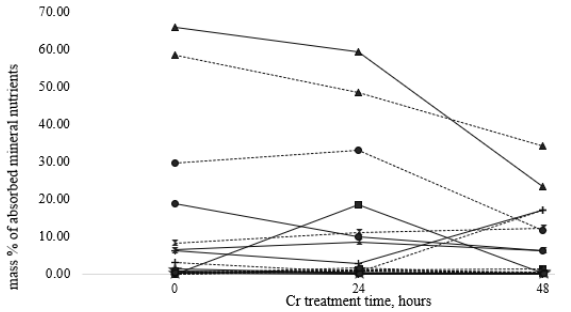

Figure 15: Mass % of absorbed selected mineral nutrients (■ Na^+,+ Mg^{2+}, ⧗ P, ▲ K^+, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ● Zn^{2+}) in *kangkong* roots after 24 and 48 h of Cr treatment under — tap water and --- hydroponics media.

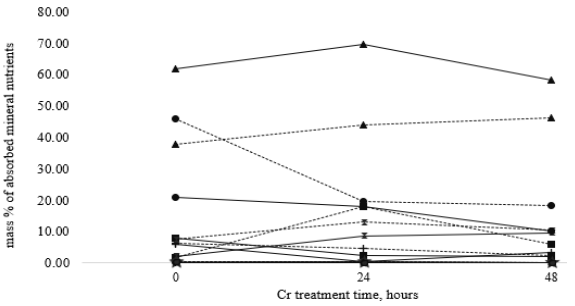

Figure 16: Mass % of absorbed selected mineral nutrients (■ Na^+,+ Mg^{2+}, ⧗ P, ▲ K^+, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ● Zn^{2+}) in *kangkong* stems after 24 and 48 h of Cr treatment under — tap water and --- hydroponics media.

As shown in Figure 15, 48 h of Cr treatment decreased the root concentrations of K^+, Ca^{2+}, Mn^{2+}, and Zn^{2+}, and increased Mg^{2+}. This trend remained the same even in the presence of nutrient supplements in the soaking medium. P, Fe^{2+}, and Cu^{2+} absorption were enhanced by the optimization of nutrients. Notably, nutrient optimization appeared to alleviate Cr interference in the absorption of P, K^+, Ca^{2+}, Mn^{2+}, Fe^{2+}, and Cu^{2+} by *kangkong* roots. Specifically, under hydroponics, P increased by 48.55% (as compared with –3.98% under the tap water), K^+ by –41.47% (as compared with –64.55% under the tap water), Mn^{2+} just decreased by –75.00% (as compared with –91.67% decrease under the tap water), and Cu^{2+} by –33.33% (as compared with –83.33% under the tap water), Fe^{2+} increased to 77.00% (as compared with –80.91% under tap water), Cu^{2+} increased dramatically by 250% (as compared with –91.67% under tap water). On the other hand, Cr treatment under hydroponics medium further reduced the uptake of Zn^{2+} as it was decreased by –88.89% as compared with –73.61% under the tap water medium. On the other hand, the nutrient supplement further increased Mg^{2+} by 452.21% compared with 171.57% under the tap water medium.

In the stems, Cr^{3+} treatment decreased Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} while it increased P (Figure 16). From the data, nutrient optimization alleviated the inhibition of Cr in the transport of K^+ and Cu^{2+}. Specifically, K^+ increased by 22.21% (compared to –5.96% under tap water), whereas Cu^{2+} just decreased by –70.00% (compared with –92.86% reduction under tap water). On the other hand, Cr treatment further reduced Mg^{2+} by –60.81% (as compared with –46.81%

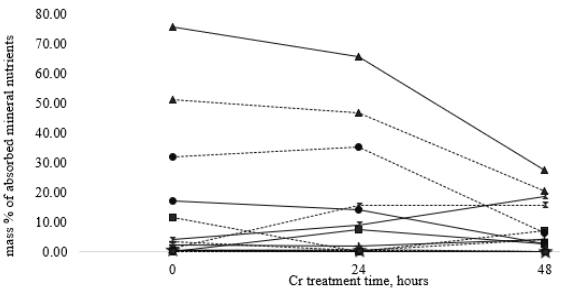

Figure 17: Mass % of absorbed selected mineral nutrients (■ Na^+,+ Mg^{2+}, ⧗ P, ▲ K^+, ● Ca^{2+}, ✖ Mn^{2+}, ⬟ Fe^{2+}, ◆ Cu^{2+}, ● Zn^{2+}) in *kangkong* leaves after 24 and 48 h of Cr treatment under — tap water and --- hydroponics media.

under tap water), Ca^{2+} by –60.07% (as compared with –50.69% under tap water), Mn^{2+} by –60.87% (as compared with –43.14% under tap water), Fe^{2+} by –69.02% (compared with –59.69 % under tap water), and Zn^{2+} by –88.24% (as compared with –59.46% under tap water). Under the hydroponics medium, P transport into the stems was decreased by –38.09% compared with its increase of 348.38% under the tap water medium.

In the leaves, Cr decreased K^+, Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} and increased Mg^{2+} and P (Figure 17). Based on the results of this experiment, nutrient optimization alleviated the restriction of Cr in the transport of Mn^{2+}, Fe^{2+}, and Zn^{2+} in the leaves. Specifically, Mn^{2+} was reduced by –66.67% (as compared to –85.19% under tap water), while Fe^{2+} decreased by –79.25% (as compared with –91.07% under tap water). Also, the transport of K^+, Ca^{2+}, and Zn^{2+} was slightly enhanced under hydroponics medium. Particularly,

K^+ was decreased by –60.03% (as compared with –63.56% under tap water), Ca^{2+} had a –80.16% decrease (as compared with –84.92% under tap water) and Cu^{2+} just declined by –91.18% (as compared with –97.86% under tap water). On the other hand, Cr treatment further reduced Zn^{2+} by –84.621% (as compared with its –55.56% decrease under the tap water). Mg^{2+} was also reduced under the hydroponics medium, i.e., 24.55% as compared with 97.17% under the tap water medium.

The Cr-treated plants showed toxicity symptoms in both tap water and the hydroponics media. While the transport of Na^+ into the leaves also decreased, visual observation showed toxicity effects of Cr in *kangkong*. This experiment suggests that Cr^{3+} induced changes in the physiological processes brought by the decreased the Ca^{2+} content in all parts as well as K^+ in the roots and the leaves of *kangkong*. Also, the low to moderate amounts of Na^+ in the stems and roots appeared to have boosted the toxic effects of Cr in *kangkong*.

It is noted that the effect of Cr in the absorption of mineral nutrients by agricultural plants is poorly documented. While the mechanism of the changes induced by Cr^{3+} in the uptake of mineral nutrients by *kangkong* could not be elaborated in the absence of similar studies in the literature, results in these experiments suggest that Cr^{3+} probably competes with Ca^{2+}, K^+, Fe^{2+}, and Zn^{2+} for the same membrane transporters. Moreover, the alterations in the transport of these nutrients probably resulted in a strong disturbance in K^+ and/or Ca^{2+} levels, which resulted in K/Ca ratio changes and could have a great impact on water balance and its corresponding plant-microbe interaction [29], [30], [44], [45].

3.7 *Statistical analysis*

With the knowledge that the heavy metal uptake and mineral nutrient absorption in various plant parts can be associated with the type of media and treatment time, a two-way analysis of variance was performed at the significant level of $P < 0.05$ was conducted.

The two-way ANOVA results indicate that the concentrations of each heavy metal and mineral nutrient under investigation are not significant between the two media types. This implies that the unknown and uncontrollable variables present in the tap water medium, such as the presence of chlorine, hard water minerals, and other nutrients would less likely affect the heavy metal uptake and mineral nutrient absorption in various plant parts vis-à-vis the hydroponics medium. However, based on treatment time, it can be observed that exposing the plant between 24 and 48 h showed significant variations on the uptake of heavy metals on the root part, especially for Pb and Cd, but no longer significant once exposed for 96 h as the plant part became saturated already. Thus, from the variables entered into the model, treatment time influences the majority of the variations in the concentration of heavy metals and mineral nutrients accumulated in various plant parts.

4 Conclusions

This study indicates that heavy metals, specifically Pb^{2+}, Cd^{2+}, and Cr^{3+}, induced changes in the absorption of mineral nutrients by *kangkong*. Most mineral ions (K^+, Ca+, Fe^{2+}, Zn^{2+}, Cu^{2+}, and Mn^{2+}) in the leaves and stems were reduced by Pb^{2+}, Cd^{2+}, and Cr^{3+} exposure, which could make *kangkong* less nutritious, aside from being toxic when consumed as food. It was also observed that these heavy metals caused a disturbance in K/Ca and K/Na ratios, which could compromise the water balance and vitality of these plants. Data also suggest that nutrient optimization can be a good strategy to alleviate the accumulation of Cd^{2+} and Pb^{2+}in water *kangkong*. The results also indicate that the mechanisms of translocation of Pb^{2+}, Cd^{2+}, and Cr^{3+} from roots to shoots behave differently in the presence of nutrients, thus more research is necessary to have a better understanding of the interactions.

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