Adv. Hort. Sci., 2024 38(4): 393-405

DOI: 10.36253/ahsc-16177
https://oaj.fupress.net/index.php/ahs

AHS
Advances in Horticultural Science

Titanium based electro-degradation of nutrient solution and green light improve autotoxicity, growth and yield of lettuce grown in recycled hydroponics

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Citation:
RAZZAK M.A., ASADUZZAMAN M., ASAO T., 2024 - *Titanium based electro-degradation of nutrient solution and green light improve autotoxicity, growth and yield of lettuce grown in recycled hydroponics.* - Adv. Hort. Sci., 38(4): 393-405

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 22 May 2024
Accepted for publication 1 August 2024

AHS - Firenze University Press
ISSN 1592-1573 (on line) - 0394-6169 (print)

Key words: Artificial lighting, controlled environment agriculture, non-renewed solution, root exudates, successive cultures.

Abstract: Lettuce grown under recycled hydroponics ensures efficient water and nutrient utilization. However, lettuce yield is often reported to be declined from successive cultures for accumulating phytotoxic root exudates. Degrading toxic exudates by titanium-based electrode and increasing photosynthetic efficiency by adding green light would improve lettuce yield. Alternate current electro-degradation (AC-ED) was applied along with addition of green light in light spectrum to enhance lettuce yield. Lettuce seedlings were grown in plant factory using half-strength of Enshi solution. Three consecutive cultures were performed under three combinations of LEDs [Red (R):Green (G): Blue (B) viz. 235:00:59, 211:30:53 and 187:60:47 µmol m^{-2} s^{-1}] using renewed (RW), non-renewed (NR) and AC-ED applied non-renewed (NR+AC-ED) nutrient solutions. Results showed that in subsequent cultures, lettuce yield declined in NR solution under 187:60:47 of R:G:B. Contrarily, NR+AC-ED solutions showed maximum lettuce growth and enhanced about 30% of yield under 30 µmol m^{-2} s^{-1} of green light addition. However, addition of 60 µmol m^{-2} s^{-1} of green light showed lower yield under all nutrient solutions. Nutritional quality of lettuce was not varied by nutrient solutions and LEDs. Our study recommends applying AC-ED for reutilizing fertigation water and addition of 30 µmol m^{-2} s^{-1} green light for higher lettuce yield under successive cultivation.

1. Introduction

Now-a-days commercial production of vegetables and fruits cultivated hydroponically are getting popularity compared to open field cultivation due to high temperatures, resurgences of insect pests and diseases as well as shortage of labor. In non-recycle system, culture solutions fully drain out to start next culture after harvesting of first culture. The damped nutrient solutions may cause environmental pollution and reduce water use efficiency from hydroponic cultivation systems. While in recycled hydroponics, nutrient solutions are reused repeatedly for several cultures by adjusting the strength of nutrient solution. Recycled hydroponics increase the water and nutrient use efficiency and avoid the cost of addition and disposal of nutrient solution. In single pass hydroponic systems, in addition to the benefits of supply management, re-collection and recycling of irrigation water or fertigation water can result in significant savings. Reuse of water is an excellent option for advanced controlled-environment agriculture (CEA) systems (Kozai *et al*., 2015). Recycling of nutrient solutions used in CEA would reduce the amount of nutrients that enter freshwater bodies like ponds, lakes, rivers etc. These benefits of recycled hydroponics are helpful for sustainable crop production by maximizing water and fertilizer use efficiency as well as reducing environmental pollution. This in turn helps achieve the targets of sustainable developmental goals in respect of clean water and sanitation (SDG 6) as well as climate actions (SDG 13).

However, in commercial cultivation, reuse of culture solutions negatively affects the yield of crop. Under this situation, farmers fully drain out single used solutions before starting new cultivation thus, requiring more water and fertilizer, ultimately increasing production cost. It is well documented that, cultures without replacement of nutrient mixture accompanies the accumulation of phytochemicals secreted by plants possibly through autotoxicity phenomena (Tang and Young, 1982; Asao *et al*., 1998; Singh *et al*., 1999; Asao *et al*., 2003; Asao *et al*., 2004; Kitazawa *et al*., 2005; Asao *et al*., 2007; Asaduzzaman *et al*., 2012), or allelopathy-like mechanism (Nakahisa *et al.*, 1994). Under plant autotoxicity, plants release chemical compounds into their rhizosphere via a variety of mechanisms like leaching, volatilization, root exudation. Asaduzzaman and Asao (2012) reported some allelochemicals in different vegetables and ornamental plants like lactic acid, benzoic acid, succinic acid, adipic acid, hydroxybenzoic acid, vanillic acid etc. To address the issue of autotoxicity, researchers have used a variety of detoxification methods for allelochemicals, like adsorption by amberlite XAD-4 (Lee *et al*., 2006) and activated charcoal (Kitazawa *et al.*, 2005), addition of amino acids (Mondal *et al*., 2013) and auxin (Kitazawa *et al*., 2007), and electro-degradation of allelochemicals (Asaduzzaman *et al*., 2012; Talukder *et al.*, 2019 a). Among all above mentioned methods, electro-degradation is the simplest and cost-effective method. The electro-degradation (ED) machine is a portable electric device that allows culture solution to pass through the titanium electrode, where electrochemical degradation occurs.

On the other hand, one of the most important factors in crop production is light. Supplying an adequate amount of artificial light is important for sustainable crop production through hydroponics in a vegetable factory. Additionally, release of growth inhibitors, such as secondary metabolites associated with photosynthesis, may be influenced by light conditions (Darko *et al*., 2014). Generally, crops are cultivated using fluorescent light in CEA. However, for higher light spectral emissions and higher amount of electricity usage of fluorescent light, now-a-days light emitting diodes (LED) is getting popularity. A narrow wavelength range of high-quality light produced by LED is suitable for plant growth and development (Carvalho and Folta, 2014). Red and blue light is commonly used in plant factories considering their higher photosynthetic efficiency. LED lights in red (660 nm) and blue (450 nm) are commonly used to grow a variety of crops including lettuce, spinach and radish (Yanagi *et al*., 1996; Hanyu and Shoji, 2002). Red and blue light, as well as their combinations, are the most effective in promoting plant growth and development and changing their architecture in the visible light spectrum (Naznin *et al*., 2019). Since the plants grown under combination of single-band blue and red light displays purplish-gray shade, it is difficult to monitor the health status of plants especially leaves with insect and disease infections. But when examined in a full spectrum of light environment by adding green light (550 nm) with red and blue, the color of the plant could be observed as green which largely improves the working conditions (Razzak *et al*., 2022; Kim *et al*., 2004). Besides that, at low photosynthetic photon flux density (PPFD), green

light has a lower quantum yield than red and blue light due to its lower absorptance, but at high PPFD, red and blue light have a lower quantum yield than green light due to uniform dispersion of the of light in lower leaves and into the plant canopy (Sun *et al*., 1998; Evans and Vogelmann, 2003; Terashima *et al*., 2009). Additionally, applying green light creates a full spectrum of white light which makes a congenial working environment in plant factory. We hypothesized that application of AC-ED and supplementation of green LED would prevent the retardation of growth and enhance the yield of lettuce. The present study was executed to investigate the effects of AC-ED in non-renewed nutrient solution for successive lettuce cultures under recycled hydroponics and also to observe the influence of supplementation of green light to red and blue light in vegetable factories.

2. Materials and Methods

Planting materials and process of lettuce cultivation

Lettuce seeds (*Lactuca sativa* cv. Souther) were sown in vermiculite (1-5 mm size) -filled cell trays and placed in a growth chamber under the following conditions: temperature: 25/20°C (day/night), relative humidity: 60%, fluorescent light intensity: 140-160 mol m^{-2} s^{-1}, photoperiod: 16 hrs. light and 8 hrs. dark , and CO_2: 1000 ppm. In the first 7 days, only tap water was supplied in the tray and from 8th day 50% Enshi nutrient solution (Hori, 1966) was added. The chemical compositions of the Enshi solution were presented in Table 1. After 21 days, seedlings were planted in a vegetable factory in three steps in vertical growing beds (125 cm × 90 cm × 10.5 cm). Each growing bed had 18 lettuce seedlings held in place by urethane cubes (23 mm× 23 mm × 27 mm). Fifty percent of the Enshi nutrient solution was pumped into three beds, each with a 50-L capacity and a 300-L saving tank. A timer (KS-1500, Iuchi, Osaka, Japan) and an automatic pump (KP-101, Koshin, Kyoto, Japan) were set to recycle the nutrient solutions at 55/5 min (recycled/stopped). Except for light conditions, the plant factory maintained similar environmental conditions to the ambient room conditions.

Table 1 - Chemical compositions of "Enshi" nutrient solution (half-strength) (Hori, 1966)

Chemicals	Amounts (g/1000 L)
$Ca(NO_3)_2.4H_2O$	475
KNO_3	405
$MgSO_4.7H_2O$	250
$NH_4H_2PO_4$	77.5
H_3BO_3	3
$ZnSO_4.7H_2O$	0.22
$MnSO_4.4H2O$	2
$CuSO_4.5H_2O$	0.05
$Na_2MoO_4.2H_2O$	0.02
Na Fe-EDTA	25

Experimental treatments and data measurement during harvest

Lettuce seedlings were planted in three types of nutrient solutions under three LED light conditions with and without addition of green light in light spectrum. The solutions were considered as renewed (R), non-renewed (NR), and NR+AC-ED (alternate current electro-degradation). There were three extents of green light supplementation to red and blue with total light intensity (µmol m^{-2} s^{-1}) as R:G:B = 235:00:59 (G0), R:G:B = 211:30:53 (G30), and R:G:B = 187:60:47 (G60) (Fig. 1 a). Plants cultivated under each lighting conditions were separated from other plants by a silver sheet which spread from light sources up to the bottom of the growth beds to avoid mix up effects of LED treatments.

The PPFD of the LED combinations were measured by MQ-200 Quantum sensor with PAR (photosynthetically active radiation) meter (Apogee Instruments, Inc. Logan, UT, USA) at five points above the plant canopy, 20 cm from the LED panels. The spectral profiles of different LED conditions employed in this experiment are presented in figure 1 (b, c, d). Electro-degradation was applied continuously during the experiment in case of AC-ED treatment. The size electric current and voltage of the electric supply was set to 1.5. For lighting with LEDs, the pulse-wide modulation at 550 Hz under 50% duty was employed. Cultivation and harvest of lettuce plants were consecutively repeated for three cycles in the above-mentioned culture solutions under LEDs. Nine plants from each replication of each treatment were sampled for comparison. Data on growth traits, and yield were measured at harvest. Relative chlorophyll contents were measured using a SPAD meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan).

Management of nutrient solution

In renewed treatment, nutrient solution was replaced by freshly prepared 50% Enshi nutrient

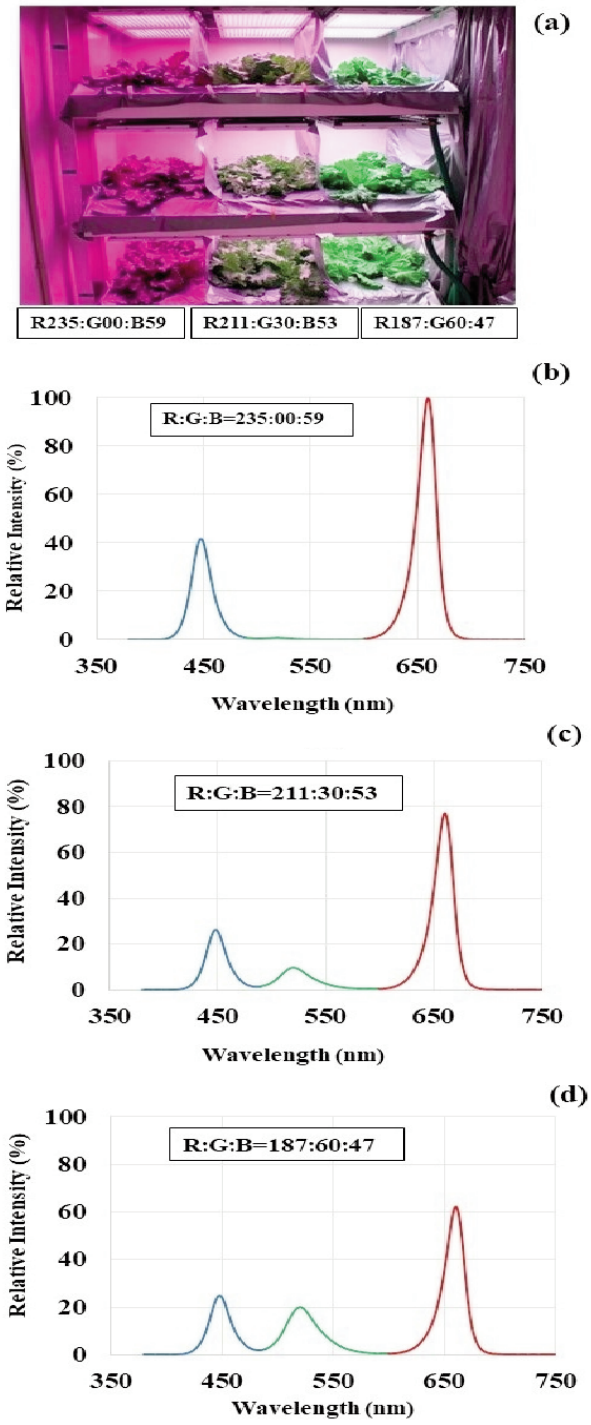

Fig. 1 - Lettuce cultivated under different combinations of red, green and blue LED lights (a) using different nutrient solutions (picture from culture cycle I) along with the spectral distribution (relative intensity) of red (R), green (G) and blue (B) light (b, c, d, respectively) used in the experiment. The wavelength of emission peaks of red, green and blue light was observed at 660 nm, 520 nm and 445 nm, respectively. The PAR meter was used to quantify the photosynthetic photon flux density (mean PPFD, µmol m^{-2} s^{-1}) of LED combinations at five sites at the plant canopy, 20 cm from the LED panels (n=4).

solution maintaining the electrical conductivity (EC) of 1.42 dSm^{-1}. On the other hand, nutrient solution was not renewed throughout the experiment from culture I to culture III in case of non-renewed and non-renewed + AC-ED treatments. All types of nutrient solutions were fresh during starting culture cycle I (CI), but solutions were once used and twice used in culture cycle II (CII) and culture III (CIII) except renewed treatment. However, almost similar EC level (1.42 dS m^{-1}) of the nutrient solutions were maintained by weekly-based supplementation of the nutrient stock solutions. The EC of the culture solution was measured (before and after nutrient adjustment) by EC meter (ES-51, Horiba, Ltd., Kyoto, Japan) and pH values were checked by pH meter (D-12, Horiba, Ltd., Kyoto, Japan) at 7 days intervals and the range observed was 7.00-7.20. The EC values of nutrient solutions during weekly supplementation of nutrient stock solutions was presented as supplementary information in figure 1S. The EC and pH of used tap water was 0.22 dS m^{-1} and 8.0, respectively.

Components of Electro-degradation system and its working principle

An AC-type electrode (Yonago Shinko Co., Ltd., Tottori, Japan) was used to degrade the accumulated autotoxic chemicals. The AC-ED electrode had a middle core made of titanium with a surface area of 53.1 cm^2 (anode/cathode) surrounded by a cylindrical tube of 95.5 cm^2 (cathode/anode) titanium. The solution was able to pass through the electrode where degradation occurred. The electrodes were connected with a digital AC power provider (AD-8735D, AND, Japan). In an electro-degradation machine, the culture solution can pass through the electrode where allelochemicals degradation take place. Different components of AC-ED system were shown in figure 2. At the anode of the electrode, autotoxic compounds are oxidized by hydroxyl radicals (OH^-) developed

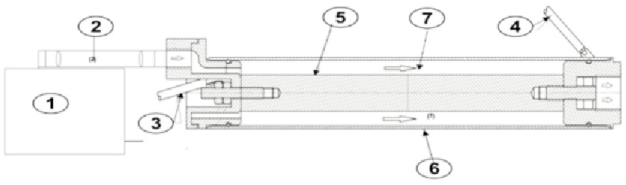

Fig. 2 - Schematic diagram of an electrode utilized in electro-degradation. There are several different parts, including a pump (1), a plastic tube linking the pump and electrode (2), an anode (3), a cathode (4), a central ferrite core (5), a titanium pipe that is cylindrical (6), and a flow of nutrient solution (7) (Asaduzzaman *et al.*, 2012).

from water and combustion of adsorbed chemicals by forming carbon dioxide (CO_2) (Fleszar and Poszyńska 1985; Comninellis and Pulgarin 1991; Feng and Li 2003).

Mineral nutrient analysis in shoots and roots

Shoots and roots that had been separated upon harvest were dried at 80°C for 72 hours in oven (DKN812, Yamato Scientific Co. Ltd., Japan), and ground into powder using a mixer (National MX-X53, Japan). Microwave-assisted digestion was performed for 0.25 g of ground materials suspended in 8 ml of 60% HNO_3 (ETHOS1, Milestone S.r.l., Bergamo, Italy). Following the digestion, the sample volume was adjusted to 50 ml by adding distilled water, and then the solution was filtered through a filter paper (Advantec Grade no. 131, 185 mm). The resultant fluid was subjected to analysis of element compositions with an atomic absorption spectrophotometer (Z-2310, Hitachi High Technologies Corporation, Tokyo, Japan).

Determination of ascorbic acid in lettuce leaves

The ascorbic acid content in leaves was determined after freezing in a freezer (at -30°C). Stored samples were removed from the freezer and pressed to extract enough juice for analysis. The ascorbic acid content was determined using spectrophotometer colorimetry with 2,4-dinitrophenylhydrazine (DNP) at 520 nm wavelength (Razzak *et al*., 2022).

Experimental design and statistical analysis

Three independent experiments were implemented following completely randomized design (CRD) of three replications in the plant factory. The collected data were analyzed for two-way ANOVA by the Statcel 4 software (OMS publication, Tokorozawa, Saitama, Japan). For mean differences Tukey-Kramar test was done at $P<0.05$.

3. Results

Yield performance of lettuce cultivated in different nutrient solutions under supplemented green light in three successive culture cycles (CI -CIII)

First culture cycle (CI)

In first culture cycle (CI), lettuce showed lower yield (shoot fresh weight- SFW) (54.1 g plant^{-1}) in NR solution when lettuce cultivated under G60 LEDs compared to the RW (70.6 and 69.5 g plant^{-1}, respectively) and AC-ED solution (66.9 and 67.4 g plant^{-1}, respectively) under the G0 and G30 LEDs (Fig. 3a). The reason for lowering yield performance might be due to the addition of higher amount of green light and also for autotoxicity in NR solution. Shoot dry weights were almost similar in treatment combinations except NR solution which was lower under G60 compared to RW solution under G30 only.

Second culture cycle (CII)

In second culture cycle (CII), it was noted that higher SFW of lettuce was found in RW (66.3 g plant^{-1}) and once used AC-ED treated (65.7 g plant^{-1}) solutions under G30 LEDs which was similar under G0 (Fig. 3b). The lower SFW were recorded in the once used NR solution under the LED of G0 (50.7 g plant^{-1}) and G60 (43.3 g plant^{-1}). In addition, shoot dry weight was declined in RW and once used NR solution under

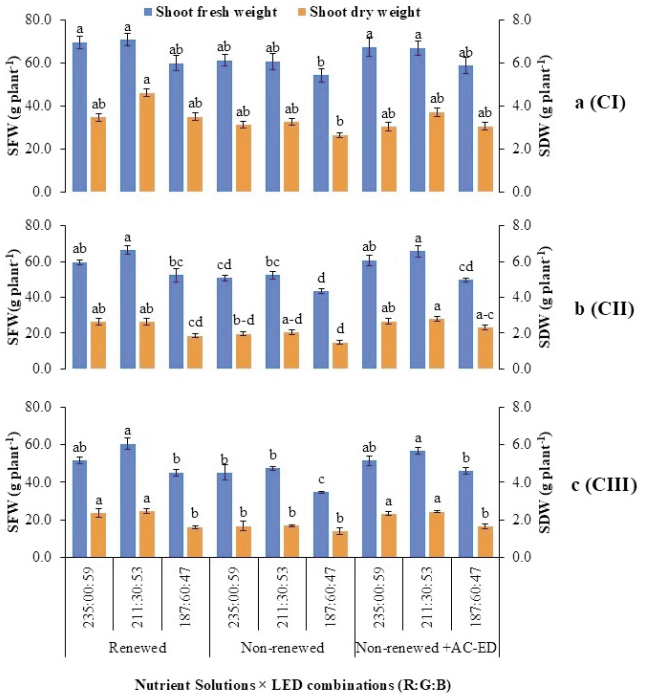

Fig. 3 - Shoot fresh weight (SFW) and shoot dry weight (SDW) of lettuce cultivated using different nutrient solutions in three culture cycles [a(CI), b(CII) and c(CIII)] under supplemented green light. Nutrient solutions were fresh at starting, once used and twice used in CI, CII and CIII, respectively. AC-ED= Alternate current electro-degradation. Photosynthetic photon flux density from R (red), G (green), and B (blue) of LED combinations (mean PPFD, µmol m^{-2} s^{-1}). Standard error of the mean (SE) is shown as a bar (n = 9). According to the Tukey-Kramar test at $P<0.05$, different letters indicate significant differences between treatments.

G60 compared to AC-ED treated solution under G30.

Final culture cycle (CIII)

In the final culture cycle (CIII), lettuce yield (SFW) was negatively affected in twice used NR solution and the lowest SFW was recorded when lettuce plants exposed to G60 LEDs (Fig. 3c). On the other hand, the higher lettuce SFW was found in both RW and twice used NR+AC-ED solution under the LED of G30 which was insignificant with the LED of G0 using the same solutions. Besides that, lower shoot dry weight was measured in RW, twice used NR and AC-ED treated solutions under G60 along with NR solutions under G0 and G30 LEDs.

Variation in leaf and root parameters of lettuce cultivated using different nutrient solutions under supplemented green light in three successive culture cycles (CI -CIII)

Leaf number and leaf relative chlorophyll content of lettuce plants showed no variation among the nutrient solutions and LED treatments in CII and CIII however, in first culture cycle (CI), lettuce plants growing in the RW solution under the G30 and G60 LEDs exhibited fewer leaves compared to some of the other treatments (Table 2). On the other hand, AC-ED treated NR solutions showed lower relative chlorophyll content under all LED conditions and NR solutions under G30 and G60 (Table 2). Additionally, in subsequent cultures (CII and CIII), leaves of plants growing in the NR solution were smaller (length and width) when exposed to the G0 and G60 LEDs and RW solutions under G60 (Table 2).

Lettuce plants developed longer roots under AC-ED treated NR solutions under all LEDs whereas RW and NR showed shorter roots under G0 and G60 LEDs in CI. But in CII and CIII RW produced smaller roots under all LEDs (Table 3). In case of root dry weight, NR solution given lower root mass under G60 in all culture cycles (CI, CII and CIII) (Table 3). Though lower root length was observed in RW solutions under all LEDs but root dry weight was higher. The reason might be producing higher root numbers with shorter length per plant.

Nutritional quality of lettuce shoots and roots cultivated using different nutrient solutions under supplemented green light in three successive culture cycles (CI -CIII)

Ascorbic acid (Vitamin C) content in lettuce leaves did not showed variation among different nutrient solutions and LEDs in 3 subsequent lettuce cultures (CI-CIII) (Table 3). Among the mineral nutrients content in lettuce shoots, Ca and Na content varied in the final culture (CIII) and Fe in CII as well as Zn in CI whereas other nutrients did not showed variation among the nutrient solutions and LEDs in different culture cycles (Table 4 a, b). Compared to RW solution under G30, Ca content in lettuce shoot was reduced in twice used NR and AC-ED treated solutions in CIII. The Fe content in lettuce shoot was determined lower in once used AC-ED treated solution under G30 and once used NR solution under G0 and G30 LEDs in CII. Moreover, Zn content in lettuce shoot in CI was declined in AC-ED solution under G0; NR solution under G0 and G30 as well as RW solution under all LED conditions.

Contrarily, in lettuce roots, Ca content in the culture cycle CI was measured minimum in NR+AC-ED solution under G60 LEDs (Table 5 a, b). However, in lettuce roots, lower K was recorded in RW solution under the G30, and G60 LEDs compared to NR solution under G60 LEDs. The Zn content in lettuce root was estimated higher in NR solution compared to other treatment combinations when lettuce plant cultivated under G60 LEDs. In the second cycle (CII), the amount of K in lettuce roots was reduced when cultivated using RW solutions by exposing to G30 and G60 LEDs compared to once used NR and AC-ED treated solutions under G60 LEDs. Additionally, Na content in roots was decreased in RW and once used AC-ED treated solutions under G0 and G30 whereas lower Fe was found in once used AC-ED treated solution under G30 and G60 LEDs. In the final culture cycle (CIII), higher amount of Ca in root was determined in NR solution under G30. However, lower K content was observed in twice used NR under G0 and AC-ED treated solutions under G0 and G60 LEDs. Comparatively lower Zn content in roots was recorded in RW solutions under all LEDs.

4. Discussion and Conclusions

In recycled hydroponic systems, crop cultivation by using same culture solutions several times is limited for developing some autotoxic chemicals which are released from plant roots. Many researchers reported such type of autotoxicity phenomenon when crop cultivated hydroponically using unchanged culture solutions in hydroponic like in lettuce (Lee *et al*., 2006), in strawberry (Kitazawa

Table 2 - Leaf characteristics of lettuce cultivated in renewed, non-renewed, and non-renewed + AC-ED applied nutrient solutions under supplemented green light in three successive culture cycles (CI -CIII)

Nutrient solutions and LEDs (R:G:B)[z]		Leaf number plant[-1]			Maximum Leaf Length (cm)			Maximum Leaf Width (cm)			Relative Chlorophyll Content (%)		
		CI[w]	CII	CIII	CI	CII	CIII	CI	CII	CIII	CI	CII	CIII
Renewed	235:00:59	13.1 a-c	10.7 a	10.7 a	20.8 ab	22.0 ab	18.5 bc	18.5 ab	17.9 bc	15.3 a-c	34.9 a	30.5 a	35.3 a
	211:30:53	12.7 bc	10.8 a	10.5 a	21.6 ab	21.0 a-d	19.2 a-c	17.9 a-c	16.6 cd	14.7 a-c	32.9 a-c	30.2 a	33.8 a
	187:60:47	11.9 c[x]	10.9 a	10.2 a	21.7 ab	19.9 c	18.4 bc	18.3 ab	16.0 d	12.2 d	32.5 a-c	33.1 a	33.4 a
Non-renewed	235:00:59	13.9 ab	10.8 a	10.7 a	19.8 b	19.5 d	17.7 c	16.4 bc	16.9 b-d	14.1 b-d	33.6 ab	30.5 a	32.9 a
	211:30:53	14.3 a	10.9 a	11.2 a	21.2 ab	20.9 b-d	18.4 bc	17.8 a-c	16.2 d	14.8 a-c	30.2 b-d	29.1 a	32.7 a
	187:60:47	13.2 a-c	10.7 a	10.0 a	19.7 b	17.8 e	16.9 c	15.6 c	14.1 e	13.6 cd	29.0 cd	33.7 a	32.0 a
Non-renewed +AC-ED[y]	235:00:59	13.4 ab	10.9 a	10.7 a	22.1 a	21.0 a-c	20.9 a	19.1 a	18.4 ab	16.8 a	28.0 d	30.3 a	31.0 a
	211:30:53	13.7 ab	11.3 a	10.8 a	22.6 a	22.5 a	21.3 a	20.3 a	19.7 a	15.8 ab	29.1 cd	30.3 a	31.5 a
	187:60:47	14.0 ab	10.5 a	10.6 a	22.3 a	22.2 ab	20.5 ab	18.0 a-c	17.1 b-d	15.3 a-c	29.8 b-d	33.7 a	32.8 a
Significance level		*	NS	NS	*	*	*	*	*	*	*	NS	NS

[w] CI (culture cycle I) = fresh nutrient solution; CII (culture cycle II)= once used nutrient solution and CIII (culture cycle III)= twice used nutrient solution.
[x] according to the Tukey-Kramar test at P <0.05, different letters in a column have substantially different, and 'ns' denotes non-significant differences.
[y] AC-ED = Alternate current electro-degradation.
[z] mean photosynthetic photon flux density (mean PPFD, µmol m^{-2} s^{-1}) from combinations of red (R), green (G), and blue (B) LEDs.

Table 3 - Root characteristics and quality of lettuce cultivated in renewed, non-renewed, and non-renewed + AC-ED applied nutrient solutions under supplemented green in three successive culture cycles (CI -CIII)

Nutrient solutions and LEDs (R:G:B)[z]		Root Length (cm)			Root Dry Weight (g $plant^{-1}$)			Ascorbic Acid (mg/100g FW)		
		CI[w]	CII	CIII	CI	CII	CIII	CI	CII	CIII
Renewed	235:00:59	22.0 b[x]	44.9 b	36.0 c	0.17 ab	0.20 a	0.18 a	9.67 a	8.5 a	9.3 a
	211:30:53	23.1 ab	44.4 b	36.2 c	0.20 a	0.17 ab	0.16 ab	7.16 a	9.6 a	10.1 a
	187:60:47	22.6 b	45.0 b	36.6 c	0.16 ab	0.12 bc	0.14 ab	8.38 a	8.5 a	11.3 a
Non-renewed	235:00:59	21.4 b	62.6 a	55.1 ab	0.15 ab	0.12 bc	0.10 ab	9.50 a	11.6 a	11.4 a
	211:30:53	24.3 ab	61.3 a	56.8 ab	0.15 ab	0.12 bc	0.10 ab	7.92 a	10.6 a	11.2 a
	187:60:47	20.1 b	54.4 ab	50.5 b	0.12 b	0.10 c	0.08 b	8.78 a	11.0 a	11.8 a
Non-renewed +AC-ED [y]	235:00:59	29.8 a	50.6 ab	61.9 a	0.16 ab	0.15 ab	0.18 a	7.23 a	9.5 a	9.7 a
	211:30:53	34.7 a	58.3 ab	53.6 ab	0.23 a	0.16 ab	0.14 ab	6.10 a	9.2 a	9.9 a
	187:60:47	26.4 ab	49.6 ab	59.9 a	0.16 ab	0.13 bc	0.12 ab	7.59 a	8.5 a	12.7 a
Significance level		*	*	*	*	*	*	NS	NS	NS

[w] CI (culture cycle I) = fresh nutrient solution; CII (culture cycle II)= once used nutrient solution and CIII (culture cycle III)= twice used nutrient solution.
[x] according to the Tukey-Kramar test at P <0.05, different letters in a column have substantially different, and 'ns' denotes non-significant differences.
[y] AC-ED = Alternate current electro-degradation.
[z] mean photosynthetic photon flux density (mean PPFD, µmol m^{-2} s^{-1}) from combinations of red (R), green (G), and blue (B) LEDs.

Table 4 a - Macronutrients content in lettuce shoots cultivated in renewed, non-renewed, and non-renewed + AC-ED applied nutrient solutions under supplemented green in three successive culture cycles (CI -CIII)

Nutrient solutions and LEDs (R:G:B)[z]		Calcium (mg/100 gFW)			Magnesium (mg/100 gFW)			Potassium (mg/100 gFW)		
		CI[w]	CII	CIII	CI[w]	CII	CIII	CI[w]	CII	CIII
Renewed	235:00:59	52.7 a	70.6 a	61.3 ab	15.6 a	15.8 a	15.9 a	405.7 a	550.7 a	462.7 a
	211:30:53	64.7 a	62.6 a	86.5 a	20.6 a	14.8 a	18.4 a	465.3 a	425.8 a	488.5 a
	187:60:47	73.3 a	57.4 a	67.8 ab	22.3 a	14.2 a	16.1 a	488.7 a	507.3 a	454.6 a
Non-renewed	235:00:59	57.8 a	51.6 a	48.6 ab	18.7 a	15.5 a	14.4 a	428 a	469.5 a	390.2 a
	211:30:53	60.1 a	57.8 a	52.5 ab	19.3 a	16.8 a	12.0 a	418.2 a	474.7 a	343.6 a
	187:60:47	55.4 a	64.1 a	39.6 b[x]	17.0 a	17.6 a	12.3 a	378.3 a	484.1 a	345.5 a
Non-renewed +AC-ED[y]	235:00:59	65.5 a	62.3 a	38.0 b	19.5 a	17.2 a	12.6 a	448.2 a	441.7 a	369.0 a
	211:30:53	85.1 a	52.0 a	62.1 ab	26.8 a	15.0 a	13.2 a	516.5 a	428.5 a	344.5 a
	187:60:47	49.1 a	54.7 a	64.7 ab	14.9 a	16.4 a	14.1 a	384.2 a	484.1 a	427.4 a
Significance level		NS	NS	*	NS	NS	NS	NS	NS	NS

[w] CI (culture cycle I) = fresh nutrient solution; CII (culture cycle II)= once used nutrient solution and CIII (culture cycle III)= twice used nutrient solution.
[x] according to the Tukey-Kramar test at P <0.05, different letters in a column have substantially different, and 'ns' denotes non-significant differences.
[y] AC-ED = Alternate current electro-degradation.
[z] mean photosynthetic photon flux density (mean PPFD, µmol m^{-2} s^{-1}) from combinations of red (R), green (G), and blue (B) LEDs.

Table 4 b - Micronutrients content in lettuce shoots cultivated in renewed, non-renewed, and non-renewed + AC-ED applied nutrient solutions under supplemented green in three successive culture cycles (CI -CIII)

Nutrient solutions and LEDs (R:G:B)[z]		Sodium (mg/100 g FW)			Iron (mg/100 g FW)			Zinc (mg/100 g FW)		
		CI[w]	CII	CIII	CI[w]	CII	CIII	CI[w]	CII	CIII
Renewed	235:00:59	7.5 a	7.9 a	6.7 ab	0.42 a	0.50 a	0.61 a	0.14 e	0.25 a	0.15 a
	211:30:53	7.7 a	6.1 a	7.8 a	0.41 a	0.39 ab	0.59 a	0.15 de	0.17 a	0.23 a
	187:60:47	7.4 a	7.0 a	6.4 ab	0.42 a	0.44 ab	0.52 a	0.19 c-e	0.20 a	0.16 a
Non-renewed	235:00:59	7.1 a	7.2 a	5.9 ab	0.50 a	0.35 b	0.37 a	0.21 b-d	0.16 a	0.22 a
	211:30:53	8.0 a	6.6 a	5.0 b	0.46 a	0.34 b	0.33 a	0.23 bc	0.19 a	0.18 a
	187:60:47	6.8 a	8.5 a	5.0 b	0.49 a	0.38 ab	0.36 a	0.24 a-c	0.26 a	0.19 a
Non-renewed +AC-ED[y]	235:00:59	7.8 a	6.7 a	5.1 b	0.48 a	0.39 ab	0.65 a	0.27 ab	0.26 a	0.20 a
	211:30:53	8.5 a	6.3 a	5.2 b	0.63 a	0.35 b	0.39 a	0.31 a	0.23 a	0.15 a
	187:60:47	6.7 a	6.9 a	5.8 ab	0.39 a	0.39 ab	0.48 a	0.19 c-e	0.23 a	0.17 a
Significance level		NS	NS	*	NS	*	NS	*	NS	NS

[w] CI (culture cycle I) = fresh nutrient solution; CII (culture cycle II)= once used nutrient solution and CIII (culture cycle III)= twice used nutrient solution.
[x] according to the Tukey-Kramar test at P <0.05, different letters in a column have substantially different, and 'ns' denotes non-significant differences.
[y] AC-ED = Alternate current electro-degradation.
[z] mean photosynthetic photon flux density (mean PPFD, µmol m^{-2} s^{-1}) from combinations of red (R), green (G), and blue (B) LEDs.

Table 5 a - Macronutrients status in lettuce roots cultivated in renewed, non-renewed, and non-renewed + AC-ED applied nutrient solutions under supplemented green in three successive culture cycles (CI -CIII)

Nutrient solutions and LEDs (R:G:B)[z]		Calcium (mg/100gFW)			Magnesium (mg/100gFW)			Potassium (mg/100gFW)		
		CI[w]	CII	CIII	CI[w]	CII	CIII	CI[w]	CII	CIII
Renewed	235:00:59	17.1 a	5.9 a	5.4 b	3.0 a	1.6 a	1.7 a	68 a-c	92.0 a-c	88.0 a
	211:30:53	16.6 a	8.6 a	7.8 ab	2.0 a	1.6 a	1.4 a	58.2 c	84.9 c	88.0 a
	187:60:47	9.9 bc[x]	8.2 a	5.3 b	3.0 a	1.8 a	1.2 a	61.3 bc	86.5 c	86.2 a
Non-renewed	235:00:59	11.9 a-c	10.6 a	11.3 ab	2.7 a	1.7 a	1.5 a	89.1 ab	88.8 bc	60.8 b
	211:30:53	8.5 bc	10.4 a	18.6 a	2.8 a	1.6 a	3.8 a	84.0 a-c	94.5 a-c	75.8 ab
	187:60:47	8.7 bc	6.8 a	6.2 b	2.5 a	1.8 a	1.7 a	94.6 a	108.8 ab	93.8 a
Non-renewed +AC-ED[y]	235:00:59	13.6 ab	4.7 a	4.6 b	3.1 a	1.7 a	1.5 a	76.5 a-c	92.0 a-c	59.2 b
	211:30:53	7.7 bc	5.3 a	7.5 b	3.7 a	1.9 a	2.6 a	74.3 a-c	103.9 a-c	76.0 ab
	187:60:47	6.9 c	5.9 a	5.5 b	3.3 a	1.6 a	1.9 a	88.6 a-c	111.9 a	64.1 b
Significance level		*	NS	*	NS	NS	NS	*	*	*

[w] CI (culture cycle I) = fresh nutrient solution; CII (culture cycle II)= once used nutrient solution and CIII (culture cycle III)= twice used nutrient solution.
[x] according to the Tukey-Kramar test at P <0.05, different letters in a column have substantially different, and 'ns' denotes non-significant differences.
[y] AC-ED = Alternate current electro-degradation.
[z] mean photosynthetic photon flux density (mean PPFD, µmol m^{-2} s^{-1}) from combinations of red (R), green (G), and blue (B) LEDs.

Table 5 b - Micronutrients status in lettuce roots cultivated in renewed, non-renewed, and non-renewed + AC-ED applied nutrient solutions under supplemented green in three successive culture cycles (CI -CIII)

Nutrient solutions and LEDs (R:G:B)[z]		Sodium (mg/100gFW)			Iron (mg/100gFW)			Zinc (mg/100gFW)		
		CI[w]	CII	CIII	CI[w]	CII	CIII	CI[w]	CII	CIII
Renewed	235:00:59	2.3 a	2.0 d	3.1 a	2.8 a	2.3 a-d	1.8 a	0.15 b	0.13 a	0.07 b
	211:30:53	2.3 a	2.8 b-d	3.5 a	3.5 a	2.6 a	1.8 a	0.17 b	0.14 a	0.08 b
	187:60:47	2.1 a	4.4 a	2.4 a	2.9 a	2.5 ab	1.1 a	0.14 b	0.17 a	0.06 b
Non-renewed	235:00:59	2.3 a	4.2 ab	2.8 a	2.5 a	2.5 a-c	0.9 a	0.16 b	0.15 a	0.08 ab
	211:30:53	2.2 a	3.2 a-d	3.3 a	2.5 a	2.5 a-c	1.6 a	0.18 b	0.17 a	0.12 a
	187:60:47	2.3 a	3.9 a-c	3.0 a	2.4 a	2.3 a-d	1.2 a	0.27 a	0.16 a	0.09 ab
Non-renewed +AC-ED[y]	235:00:59	3.0 a	2.0 d	1.8 a	3.8 a	1.9 b-d	1.1 a	0.15 b	0.15 a	0.10 ab
	211:30:53	2.3 a	2.5 cd	2.6 a	3.3 a	1.8 d	1.7 a	0.11 b	0.15 a	0.10 ab
	187:60:47	2.5 a	2.9 a-d	2.6 a	2.5 a	1.9 cd	2.1 a	0.12 b	0.14 a	0.08 ab
Significance level		NS	*	NS	NS	*	NS	*	NS	*

[w] CI (culture cycle I) = fresh nutrient solution; CII (culture cycle II)= once used nutrient solution and CIII (culture cycle III)= twice used nutrient solution.
[x] according to the Tukey-Kramar test at P <0.05, different letters in a column have substantially different, and 'ns' denotes non-significant differences.
[y] AC-ED = Alternate current electro-degradation.
[z] mean photosynthetic photon flux density (mean PPFD, µmol m^{-2} s^{-1}) from combinations of red (R), green (G), and blue (B) LEDs.

et al., 2005), in cucumber (Asao *et al.*, 1998; Yu and Matsui, 1994), in many leafy vegetables (Asao *et al.*, 2004) as well as different ornamental plants (Asao *et al.*, 2007). We observed in our research that during the initial culture (culture I-where nutrient solutions were newly prepared at starting the lettuce culture) lettuce yield was not shown distinct variation among the nutrient solutions under different LEDs (Fig. 3a) though lower leaf dimension and root dry weight were recorded in NR solution under G60 and fewer leaves was recorded in RW solution under the same LEDs (Table 2 and Table 3). However, NR+AC-ED solutions produced comparatively longer roots than NR and RW solutions under all LEDs (Table 3). From second culture, growth (leaf length, width, root dry weight) and yield performance were demonstrated declining trend in NR solutions under G60 but opposite trend was observed in case of root length and that declining trend was more prominent in that solutions and LEDs in third culture (Table 2, Table 3 and Fig. 3B). Reduction in growth some features and yield in successive lettuce cultures under different LEDs might be attributed to the accumulation of higher allelochemicals in the nutrient solutions in later cultures (culture cycle II and III) in NR solution, as solutions were not changed or renewed throughout the experimental period. Lee *et al.* (2006) reported that the intensity of allelochemicals increased due to repeated use of culture solutions. Similar results of lowering yield performance in lettuce in closed hydroponic systems using non-renewed solutions were pointed out by Talukder *et al*. (2019 b). Allelochemicals amount enhanced in frequently used nutrient solutions resulted in synergistic effects that inhibited development of plant (Inderjit, 1996). Several plant physiological processes such as photosynthesis, respiration, water and nutrient absorption, gene expression, phytochrome metabolism etc was influenced by the allelochemicals released from root exudates (Inderjit and Duke, 2003; Blum and Gerig, 2005). Allelochemical can act on oxidative damage by enhancing the activities of reactive oxygen species scavenging enzymes and augmenting the membrane lipid peroxidation levels (Baziramakenga *et al*., 1995; Politycka 1996; Yu *et al*., 2003; Ye *et al.*, 2004; Lara-Nuñez *et al*., 2006; Ye *et al.*, 2006). Besides that, autotoxic chemicals can modify genome-wide gene expression which trigger death of root cells (Bais *et al*., 2003). We observed comparatively lower root dry matter in NR solutions (Table 3). Root is the first plant parts which suffer more in allelochemical stresses in hydroponic systems. Roots damaged by autotoxic chemicals hamper nutrient and water absorption in plants. Though root dry weight was lower in case of once and twice used nutrient solutions, root length was recorded higher in those solutions compared to renewed solution. The possible reason for increasing root length might be for absorbing nutrient elements by longer roots because in some roots nutrient uptake might be affected by allelochemicals accumulated in the nutrient solutions in later cultures. These effects are ultimately responsible for lowering in shoot fresh weight and other growth parameters. In the case of LED conditions, the amount of red and blue light supplementation was greatly reduced under G60 LEDs considering LEDs of each solution, which may have hampered the photosynthetic rate. Kim (Kim *et al*., 2004) reported that plant growth was reduced by supplementing more than 75 µmol m^{-2} s^{-1} of green light. Plant growth and development enhanced under red and blue light and their combinations in the artificial lighting (Naznin *et al*., 2019). In our experiment higher performance of lettuce was observed under G30 which was similar with to G0 using RW and AC-ED treated nutrient solutions. This yield enhancement under G30 could be attributed to the involvement of green light in increasing total photosynthetic efficiency of the plant by maximizing light utilization in both upper and lower leaves. Green light penetrates the leaf layers more deeply than red or blue light, scattering into cellular parts of the leaf and increasing the photosynthetic efficiency of lower chloroplasts (Terashima *et al*., 2009; Brodersen and Vogelmann, 2010). Moreover, by incorporating green light in the light spectrum, light environment turned into white which was congenial for workers to implement the necessary management practices of lettuce plants.

For overcoming the inhibitory effect of allelochemicals in recycled hydroponics it is necessary to degrade or detoxify the accumulated chemicals in the nutrient solutions. For degrading chemicals from the solutions electro-degradation mechanism can be utilized where in the anode of electrode autotoxic chemicals can degrade into carbon dioxide (CO_2) (Fleszar and Poszyńska, 1985). Electro-degradation technique was successfully utilized in strawberry (Asao *et al*., 2008; Asaduzzaman *et al*., 2012; Talukder *et al*., 2019 a) and lettuce (Talukder *et al*., 2019 b). In subsequent

lettuce cultures (culture cycle II, III), we recorded higher growth and yield performance in AC-ED applied solution once or twice used culture solutions under G30 (Table 2, Table 3, Fig. 3B, C). The lettuce performance in this treatment was similar to renewed treatment under the same light condition. This yield recovery phenomenon might be due to the degrading the allelochemicals accumulated in the solutions by the electrode of AC-ED as well as enhancing photosynthetic efficiency under G30 for maximum use of energy from light by both lower and upper leaves.

Additionally, for producing lettuce in hydroponics, water and fertilizers requirement was lower in non-renewed solutions compared weekly renewal systems (Fig. 4, 5). However, hydroponic farmers' need to renew nutrient solution due to accumulation of autotoxic chemicals exudates from plant roots. If farmers can continuously use the nutrient solutions through overcoming autotoxicity from the hydroponic systems by using AC-ED, it would be possible to minimize the water and fertilizer requirements in hydroponics cultivation which ultimately reduce environmental risk that may occur through the release of nutrient solutions into environment.

Lettuce growth and yield performance declined in non-renewed solution cultivated under G60. Decreasing trend of yield reduction was more prominent in subsequent cultures (CII and CIII) using same nutrient solutions. AC-ED enhanced about 30 % of lettuce yield and successfully recovered the retarded lettuce growth under G30 of LEDs. There were no substantial differences in lettuce SFW between G0 and G30 in RW and AC-ED treated nutrient solutions. However, addition of higher amount of green light (G60) showed lower lettuce

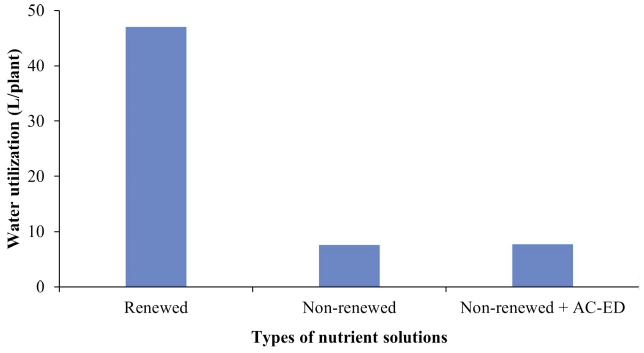

Fig. 4 - Water utilization rates (L/plant) by lettuce plants grown under different types of nutrient solutions throughout the experiment (culture cycle, CI - CIII).

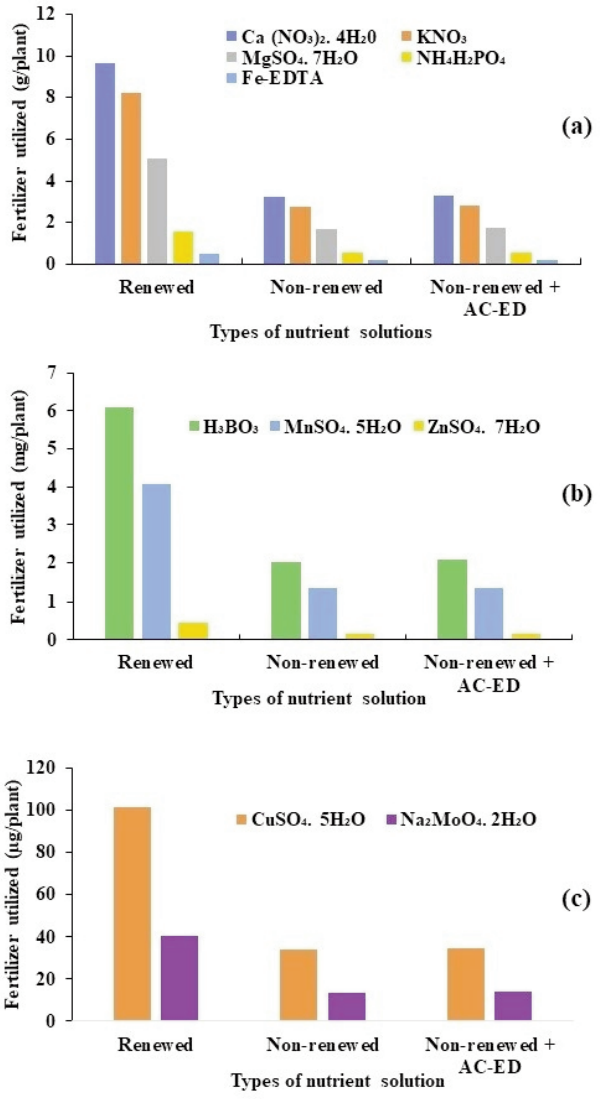

Fig. 5 - Amount of macronutrient (a) and micronutrients (b) and (c) used by lettuce plants grown under different types of nutrient solutions throughout the experiment (culture cycle CI - CIII).

performance in all types of nutrient solutions specifically once or twice used NR solutions. Therefore, AC-ED would be applied along with 30 µmol m^{-2} s^{-1} of green light supplementation for improving lettuce growth and yield cultivated in recycled hydroponics.

Acknowledgements

We would like to thank Japan Aerospace Exploration Agency (JAXA) and Faculty of Life and Environmental Sciences, Shimane University for

financial assistance to implement the research activities.

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