Comparison of digesting capacity of nitric acid and nitric acid-perchloric acid mixture and the effect of lanthanum chloride on potassium measurement

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Abstract: Nitric acid-perchloric acid mixture is the renowned digesting reagent in the scientific world of plant nutrition. Beside this, some other inorganic acids can be used as the digester of plant samples. Therefore, this experiment was conducted to find out if there is any difference between the digesting capacity of nitric acid (HNO_3) and nitric acid-perchloric acid mixture (HNO_3-$HClO_4$) or not. The hydroponic experiments were conducted with barley (*Hordeum vulgare* L. cv. Minorimugi) and rice (*Oryza sativa* L. cv. Akihikari) seedlings. At suitable stage, the plant samples were collected, washed with deionized water, separated into shoot and root, dried, grinded and then divided into two groups for shoot and root individually for two types of seedlings. One group was for only HNO_3 acid and the other group was for HNO_3-$HClO_4$ acid mixture. Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were measured after digesting the samples. There was no significant difference between the digesting capacity of HNO_3 acid and HNO_3-$HClO_4$ acid mixture. Potassium was measured by diluting the samples (200-600 times) containing lanthanum chloride ($LaCl_3$) or without $LaCl_3$. Lanthanum chloride did not have any significant effect on K measurement in this dilution system. [Nature and Science 2010;8(5):157-162]. (ISSN: 1545-0740).

***Key words**: Concentration, Lanthanum chloride, Nitric acid and nitric acid-perchloric acid mixture*

1. INTRODUCTION

Before determination of inorganic elements from plant tissues it is necessary to destroy the organic matter in plant substances. However, such a destructive method is not necessary for some inorganic ions like K, Na, Ca and Mg, which do not form any organic complex in plant tissues (Imamul Huq and Didar-ul-Alam, 2005). The methods which are used to bring about the destruction of organic matter fall into two main groups such as "wet oxidation and dry ashing". The wet oxidation includes those methods in which the destruction of organic matter is brought about by oxidation in a liquid medium, while dry ashing refers to processes in which the sample is ignited. Generally the methods of wet oxidation include the digestion of the samples with aqua regia (HCl: HNO_3 = 3:1), ternary acid mixture (HNO_3: H_2SO_4: $HClO_4$= 5:1:2), nitric acid-perchloric acid mixture (HNO_3-$HClO_4$), sulfuric acid-nitric acid mixture (H_2SO_4-HNO_3), sulfuric acid-perchloric acid mixture (H_2SO_4-$HClO_4$), nitric acid-hydrogen peroxide mixture (HNO_3-H_2O_2) and/or sulfuric acid-hydrogen peroxide mixture (H_2SO_4-H_2O_2). In the wet oxidation methods, the greater part of the oxygen (O) is required for the oxidation is supplied by the nitric acid (HNO_3). For the most efficient use of HNO_3, digestion must be carried out at a low temperature, which helps to minimize the excessive losses of the elements by evaporation in the early stages. In wet oxidation methods, $HClO_4$ acid prevents the excessive frothing which occurs frequently when HNO_3 or H_2SO_4 acid is used singly. Wet oxidation results in the conversion of the elements P, Na, K, Ca, Mg, Fe, Mn, Zn, Cu and others to proper forms for analytical determination. Some elements like chlorine (Cl) and P may be lost at the time of wet ashing as volatile compounds (Imamul Huq and Didar-ul-Alam, 2005). From the historical period, HNO_3-$HClO_4$ mixture is being used as the suitable digester in wet oxidation method for the determination of inorganic nutrient elements of plant tissues. But there are some elements [e.g. - arsenic (As)] which determination procedure may be affected by chloride (Cl^-), because of antagonistic effect between arsenite (AsO_2^-) or arsenate (AsO_4^{3-}) and Cl^-. Carbonell-Barrachina et al. (1998) found that the increasing level of As in the nutrient solution significantly decreased the Cl concentration and uptake in the root system. Therefore, at the time of As determination Cl may show antagonistic effect on As if the solution contains excess Cl. The antagonisms

between Cl^- and NO_3^-; and Cl^- and SO_4^{2-} are well known phenomenon (Mengel and Kirkby, 1987; Marschner, 1995). It seems, Cl^- reduces the uptake of different anionic ions. Therefore, there is a possibility to interfere As determination if the solution contains Cl^-. In the digested solution As may exist as arsenate (AsO_4^{3-}) anion. In this study the samples were digested with HNO_3 acid and HNO_3-$HClO_4$ mixture to find out if there is any difference between the digesting capacities of these two. This study will provide some basic information for the future to find out the effect of $HClO_4$ on As determination, because loss of As can occur in presence of high halide concentration (Frankenberger, 2002) or during determination, halide may interferes As determination. The other objective of this report was to show the effect of $LaCl_3$ on K determination.

2. MATERIALS AND METHODS

Two experiments were conducted in this study. Experiment 1 (barley plants in minus iron [-Fe] condition) was conducted to compare the digesting capacity of HNO_3 acid with HNO_3-$HClO_4$ acid mixture and to determine the effect of $LaCl_3$ on K determination. Experiment 2 (rice in plus [+Fe] condition) was conducted to verify the effect of $LaCl_3$ (purity 98% and 2% in final solution) on K determination that was obtained from the first experiment.

(1) Experiment 1:

Seed germination (Barley in -Fe condition)

Barley seeds were (*Hordeum vulgare* L. cv. Minorimugi) surface sterilized with 2% chlorinated lime [$Ca(OCl)_2$] for 45 minutes rinsed with tap water continuously for 1 hour and sandwiched between moistened towels covered with wrapping paper at 25˚C for 24 hours in electric incubator. Germinated seeds were placed on plastic net in the seed box containing 2 mM $CaCl_2$ solution in the greenhouse. After 7 days, the solution in the seed box was replaced with 1/5-strength modified Hoagland-Arnon solution containing 4.0 µM Fe^{3+}-EDTA. The plants were allowed to grow (another 7 days) until the length of the second leaf was about 20% of that of the first leaf.

Plant culture (Barley in -Fe condition)

The seedlings were transplanted after 14 days of germination (7 days in $CaCl_2$ solution and 7 days in 1/5-streangth modified Hoagland-Arnon solution) in bunches (5 plants were wrapped with sponge rubber to make one bunch). 50 bunches were placed in one bucket (capacity 35 liters) filled with ½-strength modified Hoagland-Arnon solution containing 10 µM Fe^{3+}-EDTA in the greenhouse for two days. Then the treatment was started (-Fe condition). The full-strength of modified Hoagland-Arnon solution contained 6.0 mM KNO_3; 4.0 mM $Ca(NO_3)_2$; 1.0 mM $NH_4H_2PO_4$; 2.0 mM $MgSO_4$; 20.0 µM Fe^{3+}-EDTA; 3 µM H_3BO_3; 0.5 µM $MnSO_4$; 0.2 µM $CuSO_4$; 0.4 µM $ZnSO_4$ and 0.05 µM H_2MoO_4, (Kawai et al., 1993). The –Fe medium was prepared by removing Fe^{3+}-EDTA and substituting $NH_4H_2PO_4$ with the same concentration of NaH_2PO_4 (Takagi 1993). The pH (6.5) of the solutions was adjusted daily with 1 M HCl and/or 1 M NaOH. The solutions were aerated throughout the experiment and the water level was maintained by adding deionized water. The nutrient solutions were renewed every week. The plants were grown up to 28 DAT (days after treatments). In this experiment the treatment was only –Fe modified solution of Takagi (1993). The typical photograph of –Fe experimental plants was presented in the Plate 1.

Plate 1: Typical photograph of barley seedlings at 28 DAT grown hydroponically in -Fe condition.

Heating procedure (Barley in -Fe condition)

For shoot, around 0.5 g and for root around 0.3 g samples were taken in 100 mL acid washed glass beaker. Almost 20 times HNO_3 was added for each sample and was heated at 100ºC continuously for 10 hours on electric hot plate (National Electronics Company, Japan, Model-NF-HG 59). After cooling (over night 7 hours), the samples were again heated at 140ºC for 7 hours. After cooling (over night 7 hours), additional 5 mL HNO_3 was added with one group and for other group 5 mL $HClO_4$ was added and again heated for 11 hours at 140ºC. Then the digested samples were cooled and were volume in 50 mL volumetric flask and stored in 50 mL acid washed plastic bottle.

Sample collection and preparation (Barley in -Fe condition)

The plant samples were collected and washed with deionized water three times. Shoot and root were separated with sterilized scissor and dried at 55-60˚C for 48 hours in electric oven. The oven dried plant samples were grinded properly in mortar with pestle to make it homogenous and then the samples were divided into two groups. One group was treated with HNO_3-$HClO_4$ acid

mixture (Piper, 1942) and the other group was treated with HNO_3 acid only. For each group 4 replications were used.

(2) Experiment 2:

Seed germination (Rice in +Fe condition)

Rice (*Oryza sativa* L. cv. Akihikari) seeds were surface sterilized with 2% chlorinated lime [$Ca(OCl)_2$] for 45 minutes and rinsed with tap water continuously for 1 hour. After washing, the seeds were wrapped between moistened towels and were kept in a seed growth chamber at 25˚C for 72 hours. Then the seeds were transferred on a net in a plastic seed box containing 2% $CaCl_2$ for 9 days in the greenhouse. Then the seedlings were transferred in ½-strength nutrient solution for another 9 days.

Plant culture (Rice in +Fe condition)

When the seedlings were suitable for transplantation (18 days after germination, at 3rd leaf stage of the seedlings), the treatments were started with full-strength rice solution containing 1 mM NH_4NO_3, 1 mM K_2SO_4, 0.8 mM $MgSO_4$, 0.5 mM $CaCl_2$, 0.5 mM NaH_2PO_4, 10 µM $MnSO_4$, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, 3 µM H_3BO_3, 0.05 µM H_2MoO_4 and 10 µM Fe^{3+}-citrate. 5 plants were taken in one bunch and each bucket (10 liter) containing 16 bunches. The treatments were T_1 (control, containing full-strength solution), T_2 (control + aeration), T_3 (control + 13.4 µM As), and T_4 (control + 13.4 µM As + aeration). The pH (5.5) was adjusted daily with 1 M HCl and/or 1 M NaOH at around 16.00 hours during the experiment (22.09.2004 to 01.11.2004). The solution was renewed every weak and was not aerated. Basically experiment 2 was conducted to observe the effect of aeration on As in hydroponic culture up to 21 DAT but we collected the samples to verify the result of Experiment 1. The typical photograph of +Fe rice seedlings was presented in the Plate 2.

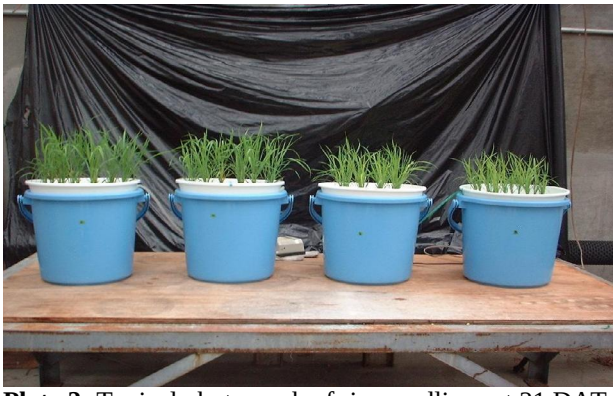

Plate 2: Typical photograph of rice seedlings at 21 DAT grown hydroponically in +Fe condition.

Sample collection and preparation (Rice in +Fe condition):

After 21 DAT, 3 bunch were taken and the plants were washed with deionized water properly and separated into shoot and root with sterilized scissor and were dried for 48 hours at 55-60˚C in electric oven. The samples were cut into small pieces suitable for digestion.

Heating procedure (Rice in +Fe condition): The individual sample was taken in acid washed 100 mL glass beaker, 3 mL analytical grade H_2SO_4 was added and covered with glass coverer and heated at 100˚C for 1.5 hours, at 140˚C for 1.5 hours and at 180˚C for 2 hours on an electric hot plate (National Electronics Company, Japan, Model NF-HG 59). After that the samples were cooled and 2 mL analytical grade H_2O_2 was added to the each sample and heated at 180˚C for 5 hours. The samples were kept for over night for cooling. In the following day another 3 mL H_2SO_4 and 2 mL H_2O_2 was added to the each samples and heated at 180˚C for 9 hours continuously. At the last stage of the digestion, all the samples were clear. After cooling, the samples were volumes at 50 mL were transferred in 50 mL acid washed plastic bottle. This extract was used for mineral elements determination.

Chemical analysis (Experiment 1 & 2):

Potassium, Ca, Mg, Fe, Mn, Zn and Cu were determined with atomic absorption spectroscopy (AAS) (Hitachi 170-30, Japan) from the digested solution. Phosphorus was determined colorimetrically using a UV-visible spectrophotometer (model UV mini 1240, Shimadzu Corporation, Kyoto, Japan) at 420 nm wavelength after developing the yellow color with vanadomolybdate as described by Barton (1948) and Jackson (1962).

Experimental design (Experiment 1 & 2):

The experiments were a completely randomized block design with 4/3 replications. Data were subjected to ANOVA. Differences between means were evaluated using a Ryan-Einot-Gabriel-Welsch multiple range test (P = 0.05) (SAS Institute, 1988) using computer origin 5 at Iwate University, Morioka, Japan.

3. RESULTS AND DISCUSSION

(1) Digesting capacity of HNO_3 acid and HNO_3-$HClO_4$ acid mixture

The concentration data of K, Ca, Mg, Fe, Mn, Zn and Cu were slightly higher or almost no change in shoot and root samples of HNO_3-$HClO_4$ digested samples as compared to only HNO_3 acid digested samples (**Fig. 1bcd and 2abcd**). However, the concentration data of P in HNO_3-$HClO_4$ digested samples were slightly lower as compared to only HNO_3 acid digested samples (**Fig. 1a**). These higher or lower values were not significant

($p<0.05$). In the case of K, Ca, Mg, Fe, Mn, Zn and Cu it could be said that, HNO_3 acid could digest the samples in better way in presence of $HClO_4$ acid as compared to without $HClO_4$ acid and gave the higher concentration data. In this experiment, slightly lower concentration of P was found in HNO_3-$HClO_4$ acid digested samples as compared to only HNO_3 acid digested samples. This may be due to the fact that in presence of $HClO_4$, oxidation of P might be little higher and evaporation loss of P might be occurred as compared to only HNO_3 acid digested samples. Phosphorus may be lost as a volatile compound at the time wet digestion (Imamul Huq and Didar-ul-Alam, 2005).

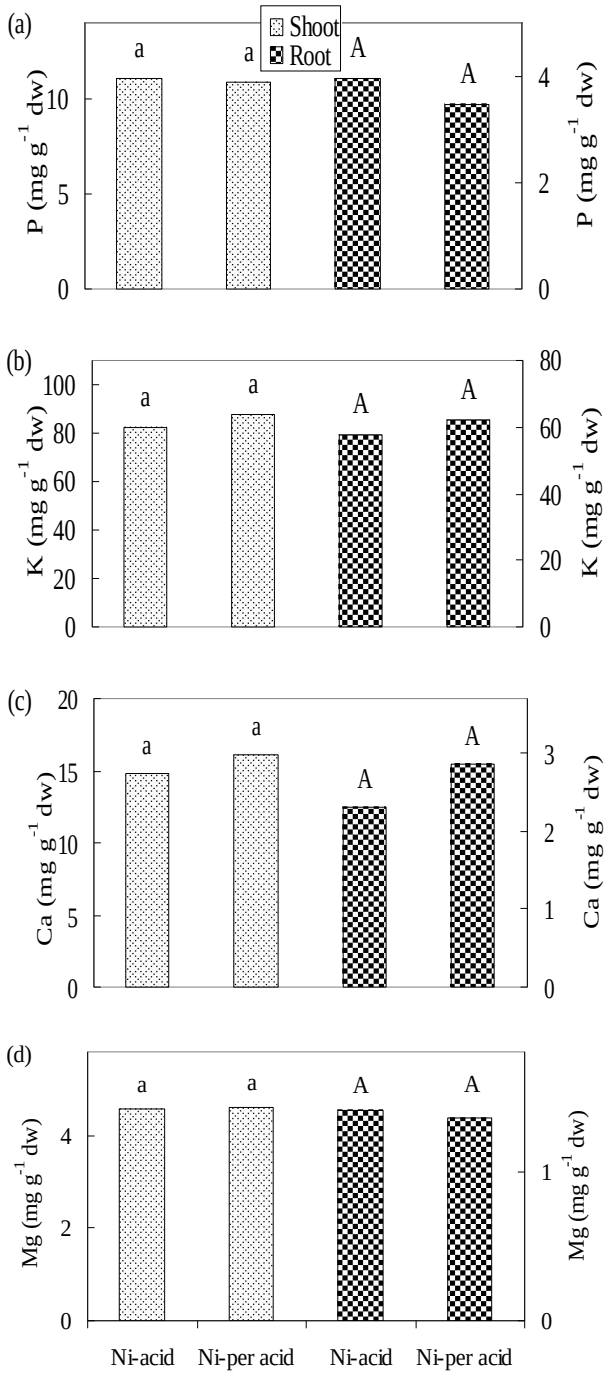

Fig. 1 Comparison of concentration of (a) P, (b) K, (c) Ca and (d) Mg of barley seedlings digested with HNO_3 acid and HNO_3-$HClO_4$ acid mixture. Bars with same letters are not are not significantly different ($p < 0.05$). Ni-acid indicates HNO_3 acid and Ni-per acid indicate HNO_3-$HClO_4$ acid mixture.

(2) Effect of $LaCl_3$ on K measurement

Potassium concentration was determined by diluting the samples 200 to 600 times with or without $LaCl_3$. Magnesium was also determined from the samples containing $LaCl_3$ both for shoot and root (data were not presented). It was observed that there was no significant difference of K concentration data between the samples of with or without $LaCl_3$ solution (**Table 1**). The concentration of K was also measured to verify the result of the first experiment from the digested samples of As containing solution. It was also found that $LaCl_3$ did not have any significant effect on K measurement (**Table 2**) and confirming the result of the first experiment.

Table 1 Concentration of K (mg g^{-1}) in presence or in absence of $LaCl_3$ of –Fe barley seedlings

	Shoot		Root	
Solution	HNO_3	HNO_3+ $HClO_4$	HNO_3	HNO_3+ $HClO_4$
Without $LaCl_3$	82.42 A	87.97 a	58.73 B	62.25 b
With $LaCl_3$	83.66 A	86.06 a	60.63 B	61.39 b

Note: Means followed by same letters in each column are not significant (p = 0.05) according to Ryan-Einot-Gabriel-Welsch Multiple Range Test.

Table 2 Concentration of K (mg g^{-1}) in presence or absence of $LaCl_3$ of +Fe rice plants

Treat.	Shoot		Root	
$LaCl_3$	without	with	without	with
T_1	47.32 a	48.85 a	32.94 A	33.09 A
T_2	49.94 b	48.37 b	34.13 B	35.55 B
T_3	41.96 c	44.38 c	29.21 C	29.48 C
T_4	45.96 d	45.97 d	34.28 D	34.32 D

Note: Means followed by same letters in each row (for shoot or root individually) are not significantly different (p = 0.05) according to a Ryan-Einot-Gabriel-Welsch Multiple Range test. T_1 (control, containing full-strength nutrient solution), T_2 (control + aeration), T_3 (control + 13.4 µM As), T_4 (control + 13.4 µM As + aeration).

(3) Effect of $LaCl_3$ on Ca measurement

The Ca concentration was also measured from the solution with or without $LaCl_3$. Higher amount of Ca was found in $LaCl_3$ containing solution both for shoot and root (**Table 3**). Shoot solution was diluted 20 times but the root solution was diluted 4 times in this case.

Table 3 Concentration of Ca (mg g^{-1}) in presence or absence of $LaCl_3$ of +Fe rice seedlings

Treat.	Shoot		Root	
DT	1	20	1	4
T_1	0.64 a	1.09 b	0.15 a	0.20 b
T_2	0.63 á	1.11 b́	0.10 á	0.22 b́
T_3	0.64 A	1.04 B	0.21 A	0.25 B
T_4	0.52 Á	1.31 B́	0.13 Á	0.30 B́

Note: Means followed by same letters in each row (for shoot or root individually) are not significantly different (p = 0.05) according to a Ryan-Einot-Gabriel-Welsch Multiple Range test. DT (Dilution times), T_1 (control, containing full-strength nutrient solution), T_2 (control + aeration), T_3 (control + 13.4 µM As), T_4 (control + 13.4 µM As + aeration).

It is well known that when the plant samples are digested with a concentrated acid digester, the inorganic elements in plants tissues are available in solution like PO_4^{3-}, K^+, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, $Zn^{2+}$and Cu^{2+}. At this condition the pH of the solution is as below as 2 and there is no precipitation of nutrient elements (Koshino, 1988). Above pH 2 (after dilution), the minerals in the solution may form complex with each other (Koshino, 1988) like Ca and Mg with P. When the diluted samples are passed through the tube of AAS, it is spilled up in presence of air pressure and reaches at the burner chamber where mist is formed in presence of acetylene gas and is burnt into flame (Koshino, 1988). In the flame, ion is transferred into atom. The samples containing Ca^{2+}, Mg^{2+}, PO_4^{3-} and SO_4^{2-} may form complexes like- $CaSO_4$, $Ca_3(PO_4)_2$, $MgSO_4$ and $Mg_3(PO_4)_2$. Thermal dissociation is one of the complex phenomenon occurred in the flame of atomic absorption without $CaSO_4$, $Ca_3(PO_4)_2$, $MgSO_4$ or $Mg_3(PO_4)_2$. After adding 2% $LaCl_3$ with the solution, $LaCl_3$ may react with SO_4^{2-} and PO_4^{3-} of Ca and/or Mg and may form insoluble complex like $La_2(SO_4)_3$ and $LaPO_4$. As a result Ca and or Mg may be free from those anions and the atoms could be measured. May be K does not form any complex with SO_4^{2-} or PO_4^{3-} in the flame and therefore, $LaCl_3$ may not have any effect on K measurement. May be $LaCl_3$ could free Ca from the insoluble complex as a result; higher Ca concentration was recorded in $LaCl_3$ treated samples (**Table 3**).

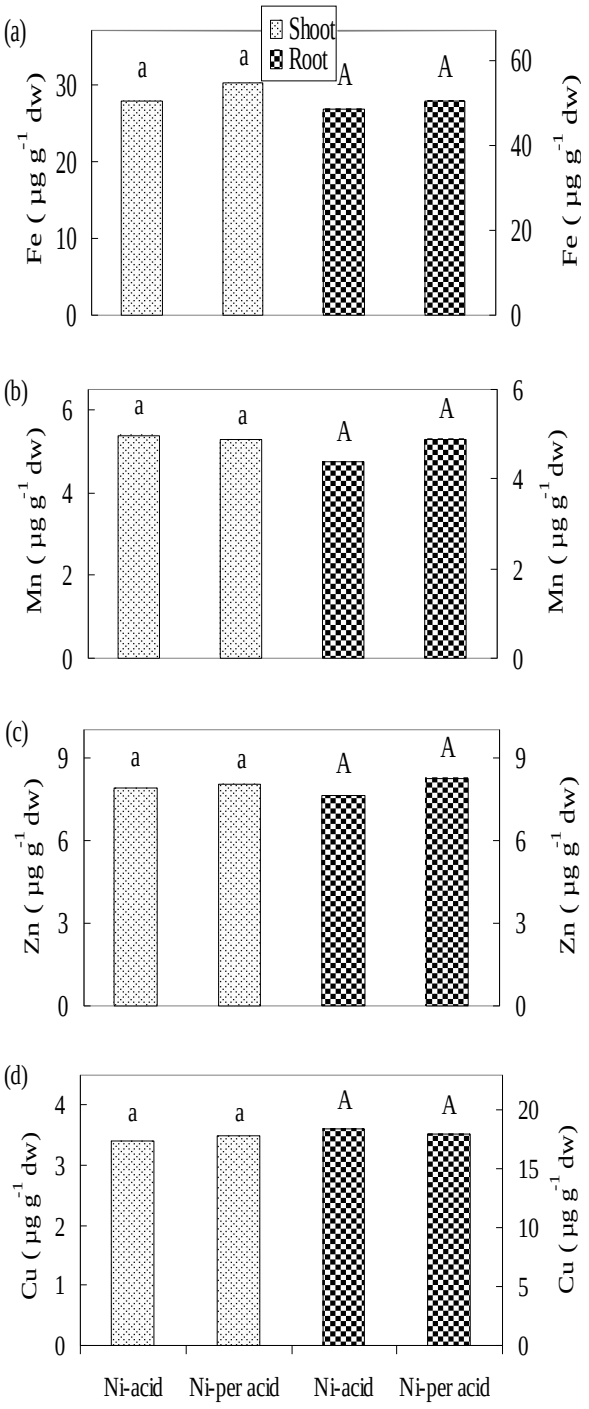

Fig. 2 Concentration of (a) Fe, (b) Mn, (c) Zn and (d) Cu of barley seedlings digested with HNO_3 acid and HNO_3-$HClO_4$ acid mixture. Bars with same letters are not are not significantly different (p < 0.05). Ni-acid indicates HNO_3 acid and Ni-per acid indicate HNO_3-$HClO_4$ acid mixture.

After adding $LaCl_3$ with the acid extract the probable reaction may be as follow

$Ca_3(PO_4)_2 + 2LaCl_3 ====== 2LaPO_4 + 3CaCl_2$
$Mg_3(PO_4)_2 + 2LaCl_3 ====== 2LaPO_4 + 3MgCl_2$
$3CaSO_4 + 2LaCl_3 ====== La_2(SO_4)_3 + 3CaCl_2$
$3MgSO_4 + 2LaCl_3 ====== La_2(SO_4)_3 + 3MgCl_2$
(Insoluble) (Soluble) (Insoluble) (Soluble)

4. CONCLUSION

The results of the present study showed that there were no significant differences between the digesting capacities of HNO_3 acid and HNO_3-$HClO_4$ acid mixtures. With the present heating procedure only HNO_3 acid could be used for the digestion of plant samples especially which contains elements, which determination could be affected by $HClO_4$. Moreover, we found less P in HNO_3-$HClO_4$ acid digested samples as compared to only HNO_3 acid digested samples. Therefore, it was suggested that only HNO_3 acid is a good digester for P and may be for As containing samples also. It was also revealed that there was no significant difference of K concentration data between the solutions containing $LaCl_3$ or without $LaCl_3$ for both experiments. Therefore, it is suggested that the K can be measured from the $LaCl_3$ containing solution especially when the mother solution was diluted enough in this experiment. In this laboratory, K and Mg could be measured from the same solution if the solution is diluted appropriately.

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