DETERMINATION OF ESSENTIAL AND TOXIC HEAVY METALS IN BARLEY (Hordeum vulgare) CULTIVATED IN SELECTED PLACES IN AMHARA REGION, ETHIOPIA

Abebaw Shitahun[1*] and Merid Tessema[2]
[1]Debre Markos University, Ethiopia, 2Addis Ababa University, Ethiopia
*Corresponding Author E-Mail: abebawshitahun@gmail.com

ABSTRACT

Barley (*Hordeum vulgare*. L) is used as food, for feeding animals and for malting in the world and in Ethiopia. The levels of essential metals (K, Mg, Ca, Na, Zn, Fe, Ni, Mn, Cu, Cr and Co) and toxic heavy metals (Pd and Cd) in barley samples collected from East Gojjam, Awi zone and West Gojjam, Ethiopia, were determined by flame atomic absorption spectrometry. For the determination, first 0.5 g barely sample was digested using mixtures of 2 mL of HNO_3, 1 mL of $HClO_4$ and 1 mL of H_2O_2 at 210 ^oC for 2:30 hr based on the optimized wet digestion procedure. The optimized wet digestion procedure was evaluated using standard addition (spiking) method and an acceptable percentage recovery was obtained. The mean metal concentration (mg/kg dry weight basis) ranges in barley are : K (4094–12238), Mg (827–983), Ca (320–886), Na (49.2–62.3), Zn (33.7–76.7), Fe (15.7 – 106), Ni (3.78–27.6), Mn (8.83–13.7), Cu (5.83–10.5), Cr (4.33–6.11), Co (3.38–5.83), Pb (1.44–2.33) and Cd (0.98–1.55). The results were compared with values reported in the literature. *[African Journal of Chemical Education—AJCE 10(1), January 2020]*

INTRODUCTION

Cereals (teff, wheat, maize, sorghum and barley) are the core of Ethiopia's agriculture and food economy which dominate crop production [1]. From these cereal crops barley is a short-season, early maturing crop with high yield potential and a wide range of adaptation. Worldwide, it can be grown successfully where other grain crops are poorly adapted, including high latitude and high elevation regions and even bordering desert. Barley is one of the founder crops of old world agriculture and was one of the first domesticated cereals [2]. Barley (*Hordeum vulgare* L.) is a multipurpose plant cultivated since ancient time for food, feeding animals, medicinal purposes and malt of alcoholic beverages [3].

In Ethiopia, barley is produced mainly for human consumption and is one of the most important food crops. [4]. Among the traditional foods prepared from cereals, some recipes such as *besso, zurbegonie* and *chiko* have a long shelf life and can only be prepared from barley grain. Other barley recipes such as *genfo*, *kolo* and *kinche* are the most popular but they can be prepared from other cereals also. Traditional foods such as *bread*, *injera*, *kitta*, *atmit* (soup) or *muk* can be prepared with only barley or blended with other cereal flours [5]. In general almost all of the important cereal based traditional recipes, with the exception of *nifro*, can be prepared with barley [5, 6]. For diabetic patients bread barley is preferred to wheat bread.

Food produced from barley is a good source for many nutrients such as protein, fiber, minerals, and B-vitamins [7]. Whole barley grain consists of about 65-68% starch, 10-17% protein, 4-9% β-glucan, 2-3% free lipids and 1.5–2.5% minerals [6, 8].

The metals 'K, Mg, Ca, Na, Zn, Fe, Ni, Mn, Cu, Cr and Co' are essential for plant and human nutrition; small amounts are required in the human diet. However, excessive amounts of any essential metals can produce toxic effects. Other metals, for example Pb, Cr (VI) and Cd are

not essential and exposure to even small amounts can produce toxic effects to all life-forms [9, 10]. Therefore the nutritional values of barley need to be investigated. This study is aimed to determine essential and toxic heavy metal in barley.

EXPERIMENTAL

Sample Collection

The samples of barley were collected from different barley growing places in Ethiopia, namely; East Gojjam, Awi zone and West Gojjam which are all located in Amhara region about 295, 452 and 563 km north-west of Addis Ababa respectively. Barley samples cultivated between June and September were collected from local markets. 1 kg of sample was purchased from different farmers and then the samples were mixed and homogenized and then 1 kg of the mixed subsample was taken. This subsample was collected using plastic bags in order to minimize contamination [3]. This procedure was also applied for other sampling sites. Finally, the collected samples were brought to the laboratory for further treatment.

Equipments

A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) was used to weigh barley samples. Volumetric flask (100 and 50 mL), measuring cylinder, filter paper (Whatman ≠42) and funnel were also used. Grinder was used to ground the sun dried barley sample. Pore size of 0.425 mm sieve was used to separate ground barley samples based on size. Plastic bags were used to store ground and raw samples to prevent metal contamination. 100 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered barley sample. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample till analysis. Buck scientific model 210 VGP (East Norwalk,USA)

atomic absorption spectrophotometer was used for the analysis of the analyte metals using air-C_2H_2 flame.

Chemicals and reagents

All solutions were prepared using analytical grade reagents: Nitric acid (65% Merch, Germany), hydrochloric acid (37% Riedel-de Haen, Germany), hydrogen peroxide (30 - 36% Scharlau, European Union), deionized water and stock standard solution of each metal were used. The calibration standard solutions were prepared using distilled and deionized water immediately before use.

Sample preparation

Each of the barley samples was thoroughly washed with tap water and then after rinsed with deionized water to remove surface contaminants like soil, dusts and spray residues. The washed barley sample was then collected with plastic bags and allowed to dry in the sun. The sun dried barley sample was ground into fine particles using a sample grinder and was stored in washed plastic bags for digestion.

Optimization of digestion procedure

Various digestion procedures were tested to digest barley samples using wet-digestion method. In this work three variables were optimized. These are: volume of the reagents for minimum consumption of chemicals, the most possible lower temperature (energy) and shorter time for the digestion of barley samples. From the results obtained, the wet digestion procedure was optimized to be 4 mL reagent volume (mixtures of 2 mL of HNO_3, 1 mL of $HClO_4$ and 1 mL of H_2O_2), temperature (at 210 ^oC) and time (for 2:30 hr) [11].

Sample digestion

According to the optimized wet digestion procedure, 0.5 g of powdered and homogenized barley sample was digested. After digestion, 2 % of 10 mL nitric acid was added to dissolve the precipitate formed on cooling and gently swirled to reduce the formation of the precipitate. The cooled digested sample was then filtered into a 50 mL standard volumetric flask with Whatman filter paper in order to remove any suspended or turbid matter. This was followed by subsequent rinsing of the filtrate with 5 mL deionized water until the volume becomes half of the total volume. Then 1% (w/v) lanthanum nitrate was added in order to make some metals free and the flask containing the sample was filled to the mark. Triplicate digestion was carried out. The digested and diluted sample solutions were then stored in tightly capped polyethylene bottles and kept in a refrigerator until analysis.

Sample analysis

Determination of the levels of metals in the digested and diluted samples was carried out with flame atomic absorption spectrometry (FAAS). First the instrument was calibrated using metal standard solutions. To calibrate the instrument, standard solutions of K, Mg, Ca, Na, Zn, Fe, Ni, Mn, Cu, Cr, Co, Pb and Cd were analyzed with FAAS (Buck Scientific Model 210GP) equipped with standard air-acetylene flame system after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument[11]. All the thirteen metals in the barley samples were determined by absorption/concentration mode and the instrument readout was recorded. The same analytical procedure was employed for the determination of metals in blank solutions too.

Validation of the optimized procedure

The efficiency of the optimized procedure is checked by analyzing certified standard reference metal solutions and spiking sample with known concentration of the analyte [12]. The spiked samples were prepared by adding a small known quantity of metal standard solution to the samples. The spiked samples were then digested applying similar digestion procedure and analyzed for the essential and toxic heavy metals [11]. Finally the percent recovery was calculated as:

$$\% R = \frac{C_M \text{ spiked} - C_M \text{ non spiked}}{C_M \text{ added for spiking}} x\ 100\%$$

Where, C_M = concentration of metal of interest

R = recovery

RESULTS AND DISCUSSION

The instrument was calibrated using four series of working standards which were prepared by diluting the intermediated standard solutions (10 mg/L). Fresh working standard solutions and the resulting correlation coefficient of the calibration curve for each metal is shown in table 1.

Table 1: Intermediate standards, working standards and correlation coefficients of the calibration curve

Metals	Concentration intermediate standard (mg/L)	Concentration of working standard (mg/L)	Correlation coefficient of the calibration curve
K	10	0.25, 0.5, 0.75, 1.5	0.99985
Mg	10	0.25, 0.5,1,2	0.99997
Ca	10	0.5, 1, 2, 4	0.9993
Na	10	0.25, 0.5,1,2	0.999999
Zn	10	0.1, 0.2, 0.4, 0.8	0.99976
Fe	10	0.5, 1, 1.5, 3	0.99954
Ni	10	0.25, 0.5, 1, 2	0.99971
Mn	10	0.25, 0.5, 1, 2	0.99999
Cu	10	0.5, 1, 1.5, 3	0.99992
Cr	10	0.5, 1, 1.5, 3	0.99991
Co	10	0.25, 0.5, 1, 2	0.99999
Pd	10	1, 2, 3, 4	0.99993
Cd	10	0.25, 0.5, 1, 2	0.99998

Percentage recoveries

The percentage recoveries for the studied metal nutrients were within the acceptable range 90-108.3. Therefore, this verifies the optimized digestion procedure was valid. The lower recovery of Cd may be attributed to the strong matrix analyte interaction.

Table 2: Recovery for the optimized procedure

Metals	Conc. in sample (mg/kg)	Amount added (mg/kg)	Conc. in spiked sample (mg/kg)	Amount recovered (mg/kg)	Recovery (%)
	Mean ± SD		Mean ± SD	Recovered ± SD	%R ± RSD
K	11000 ± 34	610	11600 ± 50	600 ± 60.5	98.4 ± 9.9
Mg	11100 ± 10.9	166	11251 ± 1.9	151 ± 11.8	90.9 ± 7.1
Ca	2600 ± 5	64	2660 ± 1.5	60 ± 5.2	93.8 ± 8.2
Na	261 ± 1	12.2	273 ± 0.7	12 ± 1.2	98.36 ± 9.8
Zn	86.2 ± 0.2	13.4	101.1 ± 0.5	14.9 ± 0.54	111.1 ± 4
Fe	46 ± 0.22	12.6	57.7 ± 0.16	11.7 ± 0.27	92.9 ± 2.1
Ni	30 ± 0.26	12	43 ± 0.26	13 ± 0.37	108.3 ± 3.1
Mn	38 ± 0.29	6.8	44.2 ± 0.26	6.2 ± 0.39	91.2 ± 5.7
Cu	11.4 ± 0.16	4	15 ± 0.24	3.6 ± 0.28	90 ± 7.2
Cr	14 ± 0.17	3	16.8 ± 0.14	2.8 ± 0.22	93.3 ± 7.3
Co	11.84 ± 0.03	2.4	14 ± 0.14	2.16 ± 0.14	90 ± 5.9
Pb	29.3 ± 0.04	0.62	30 ± 0.06	0.7 ± 0.07	112.9 ± 11.2
Cd	7.46 ± 0.01	0.62	8 ± 0.03	0.54 ± 0.03	87.1 ± 5.1

Levels of metal content in the analyzed barley samples

The concentrations of thirteen elements (K, Mg, Ca, Na, Zn, Fe, Ni, Mn, Cu, Cr, Co, Pb and Cd) in the digested barley samples are shown in Table 3 with their respective % RSD.

Table 3: Average concentration (mean ± SD, n = 9, mg/kg on dry weight basis) and relative standard deviation (% RSD) of major, minor and toxic metals in barley samples from East Gojjam, Awi Zone and West Gojjam

Metals	East Gojjam barley sample (mg/kg)		Awi Zone barley sample (mg/kg)		West Gojjam barley sample (mg/kg)	
	Mean ± SD	% RSD	Mean ± SD	% RSD	Mean ± SD	% RSD
K	4094±52.7	1.3	6071 ± 166	2.7	12238 ± 180	1.5
Mg	827 ± 50	6	905 ± 33.3	3.7	983 ± 60.1	6.1
Ca	320 ± 7.2	2.3	886 ± 11.2	1.3	427 ± 6.71	1.6
Na	62.3 ± 0.93	1.5	51.6 ± 2.90	5.6	49.2 ± 4.99	10.1
Zn	33.7 ± 0.51	1.5	76.7 ± 0.18	0.23	36.9 ± 0.22	0.6
Fe	15.7 ± 1.22	7.8	56.6 ± 2.79	4.9	106 ± 7.63	7.2
Ni	14.9 ± 0.87	5.8	27.6 ± 0.71	2.6	3.78 ± 0.33	8.7
Mn	11.2 ± 0.67	6	13.7 ± 0.44	3.2	8.83 ± 0.60	6.8
Cu	5.83 ± 0.53	9.1	10.5 ± 0.93	8.9	9.72 ± 0.87	8.9
Cr	4.33 ± 0.441	10.17	5.8 ± 0.5	8.7	6.11 ± 0.50	8.2
Co	3.38 ± 1.09	9.7	4.72 ± 0.49	10.3	5.83 ± 0.53	9.1
Pb	2.11 ± 0.23	10.9	1.44 ± 1.4	9.8	2.33 ± 0.25	10.7
Cd	1.2 ± 0.12	10	1.55 ± 0.17	11	0.98 ± 0.11	11

As it can be seen from Table 3 and Figure 1 the levels of metals differ significantly among each other and there is slight difference between the same metals from different sampling area. This is because mineral uptake in plants is a function of mineral concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants [13].

The most abundant macro elements are K (4094 - 12238 mg/kg), Mg (827-983 mg/kg) followed by Ca (320 - 886 mg/kg). The high levels of K and Mg in barley is probably due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue. It is also believed that if the soil used for cultivation of crops is treated with fertilizer and organic matter, crops would have high amount of potassium, calcium and magnesium [13]. The same reason would be the case for the presence of high amount of K, Mg and Ca in this study.

In the investigated barley samples the most accumulated microelement is Fe (15.7-106 mg/kg) followed by Zn (33.7- 76.7 mg/kg) and Ni (3.78 - 27.6 mg/kg). High Fe levels in the barley sample may be attributed to the availability of this micronutrient metal in soils of the farmland. The levels of other essential trace metals detected in barley were Mn (8.83 - 13.7 mg/Kg), Cu (5.83 - 10.5), Cr (4.33 - 6.11 mg/kg), Co (3.38 - 5.83 mg/kg), and non-essential heavy metals Pb (1.44 - 2.33), and Cd (0.98 -1.55 mg/kg). The level of Cd was the least among the metals determined. In general the concentration pattern of metals in barley was in the order:

K > Mg > Ca > Fe >Zn > Na > Ni > Mn > Cu > Cr > Co > Pb > Cd

As it can be seen from Table 3 barley can be a good source of major and minor metals that are essential to humans in addition to its food purpose. The level of Cd in barley in the present study is above the permissible limit set by FAO/WHO. According to FAO/WHO, the concentration of Cd should be (0.21 mg/kg) and lower to be safe to eat plants. The small amount of Cu, Cr and Co found in barley does not contradict with the requirement of the metal for proper functioning of the body, because these metals are required in small amounts.

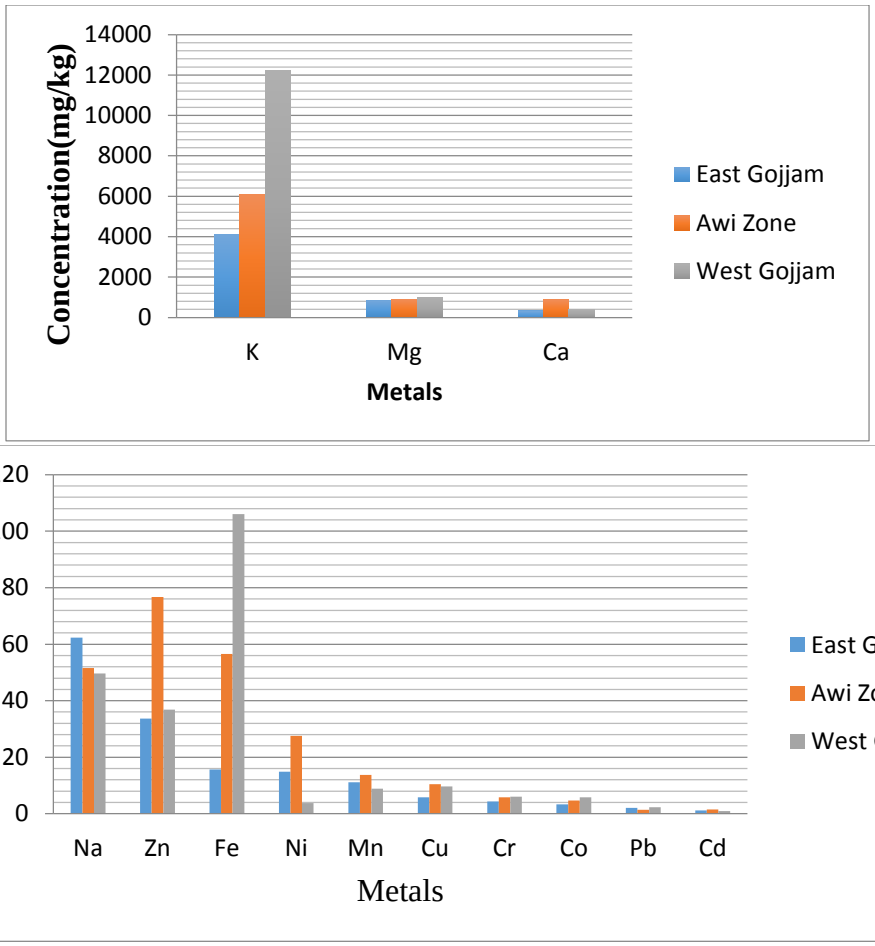

Figure 1: Distribution pattern of macro and micro elements in barley sample

Comparison of the levels of metals in barley with literature values

Comparison of the metal concentration in barley determined in this study with reported values of other researchers are presented in Table 4. As shown, levels of K, Cd, Ni and Cr in barley determined in this study was a bit higher than reported values but the Mg, Na, Pb and Mn content in barley determined in this study was lower than reported values. The level of Ca, Zn, Fe, Cu and Co in barley determined in this study was comparable with reported values.

Table 4: Comparison of determined metals concentration in barley with reported values

Metals	Analyzed (mg/kg)	Reported (mg/kg)	References
K	4094 - 12238	4719-9515	[14]
Mg	827 - 983	1157-2571	[14]
Ca	320 - 886	400 - 460	[15]
Na	49.2-62.3	893-2312	[14]
Zn	33.7- 76.7	32.9 - 35.7	[15], [16]
Fe	15.7-106	84 – 104	[15]
Ni	3.78-27.6	0.56-1.23	[17]
Mn	8.83-13.7	21.93-38.69	[17]
Cu	5.83 - 10.5	3.7	[16]
Cr	4.33 - 6.11	0.83-1.22	[17]
Co	3.38 -5.83	1.66-44.1	[17]
Pb	1.44 - 2.33	2.9	[16]
Cd	0.98 -1.55	0.62	[16]

IMPLICATIONS FOR CHEMICAL EDUCATION

Teaching learning process should be supported and referred to results of research works in chemistry. Bringing such practical works and research results to the chemistry classroom has a large contribution to the quality of chemical education. Some of the area in which this research work helps us to teach students are spectroscopic methods of analysis, metallic nutrients in crops, ways of expressing experimental results and levels of metallic nutrients in barley in the research area.

CONCLUSION

The level of essential (K, Mg, Ca, Na, Zn, Fe, Ni, Mn, Cu, Cr and Co) and non-essential (Pb and Cd) metals in barley cultivated in (East-Gojjam, Awi-zone and West-Gojjam), Ethiopia were determined by flame atomic absorption spectrometry. The level of metals in barley

determined in this study was in the following range K(4094 –12238 mg/kg), Mg (827–983 mg/kg), Ca (320–886mg/kg), Fe (15.7–106 mg/kg), Zn (38.5–55.2 mg/kg), Na(15.2–62.3 mg/kg), Ni (3.78–27.6 mg/kg), Mn (8.83–13.7 mg/kg), Cu(5.83–10.5 mg/kg), Cr (4.33–6.11 mg/kg), Co (3.38–5.83 mg/kg), Pb (1.44–2.33 mg/kg), Cd (0.98–1.55 mg/kg). The results of this work show that barley can be a good source of major and minor metals that are essential to humans in addition to its food purpose. The results show the level of Cd in barley in this study is above the permissible limit set by FAO/WHO.

REFERENCES

1. Taffesse, A. S.; Dorosh, P.; Asrat, S. Crop production in Ethiopia: regional patterns and trends, development strategy and governance division. *International Food Policy Research*, **2011**, 1, 1-32.
2. Australian government, office of the gene technology regulator. *Biology of hordeum vulgareL. (barley)*, **2008**, 1, 1-44.
3. Eticha T. and Hymete A.; Determination of Some Heavy Metals in Barley Locally Grown for Brewing and It ' s Malt in Ethiopia," *J. Bioanal. Biomed*, **2015**, vol. 7, no. 5, pp. 171–173.
4. Bantayehu, M. Analysis and correlation of stability parameters in malting barley**.** *African Crop Science Journal*, **2009**, 17, 145 -153.
5. Grando, S.; Macpherson, H. G. food barley; importance, uses and local knowledge. *International center for agricultural research in the dry area*, **2005**, 1, 1-168.
6. Shewayrga, H.; Sopade, P. A. Ethnobotany, diverse food uses, claimed health benefits and implications on conservation of barley landraces in North Eastern Ethiopia highlands. *Journal of Ethnobiology and Ethnomedicine*, **2011**, 7, 1-15.
7. Newman, R. K.; Walter, C. barley history: relationship of humans and barley through the ages. *Barley for Food and Health*: *Science*, *Technology*, *and Products*, **2008**, 1, 1-17.
8. Baik, B. K.; Ullrich, S. E. Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Science*, **2008**, 48, 233-242.
9. Tegegne, W. A. Assessment of some heavy metals concentration in selected cereals collected from local markets of Ambo, *J. Cereal. Oilseeds,* **2015**, vol. 6, no. 188, pp. 8–13.
10. Goyer, R.; Golub, M.; Choudhory, H.; Hughes, M.; Kenyon, E.; Stifelman, M. Issue paper on the human health effects of metals, **2004**, 1, 1-48.
11. Leshe, S. and Tessema, M. Determination of levels of essential and toxic heavy metals in lentil (lens culinaris medik) by flame atomic, *AJCE*, **2014**, vol. 4, pp. 16–34.
12. Mitra, S. *Sample Preparation Techniques in Analytical Chemistry*, John Wiley and sons, Inc., Hoboken, **2003**, 227-244.

13. Jung, M. C. Heavy metal concentrations in soils and factors affecting metal uptake by plants in the vicinity of a Korean Cu-W mine, *Sensors*, **2008**, 8, 2413-2423.
14. Shar, G.Q.; Kazi, T. G.; Sahito, S.; Arain, S.A. Estimation of essential macro-nutrients in sixteen barley cultivars (Hordeum Vulgare L.) and its soil, ground at wheat research station Tandojam, Sindh. *Journal of Chemical Society of Pakistan*, **2005**, 27, 69-72.
15. Abebe, Y.; Bogale, A.; Hambidge, K. M.; Stoecker, B. J.; Bailey, K.; Gibsond, R. S. Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability. *Journal of Food Composition and Analysis*, **2007**, 20, 161-168.
16. Chlopecka, A. Forms of trace metals from inorganic sources in Soils and amounts found in spring barley. *Water, Air, and Soil pollution*, **1993**, 69, 127-134.
17. Shar, G. Q.; Kazi, T. G.; Jatoi, W. B.; Makhija, P. M.; Sahito, S. B.; Shar, A. H.; Soomro, F. M. Determination of Heavy Metals in Eight Barley Cultivars Collected from Wheat Research Station Tandojam , Sindh , Pakistan, *Pak. J. Anal. Environ. Chem*, **2013**, vol. 14, no. 1, pp. 47–5