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ESSENTIAL AND NONESSENTIAL METAL CONCENTRATIONS IN MOREL MUSHROOM (*MORCHELLA ESCULENTA*) IN DIR-KOHISTAN, PAKISTAN

JUMA MOHAMMAD[1,2*], SARDAR KHAN[1*], MUHAMMAD TAHIR SHAH[3], ISLAM-UD-DIN[4] AND ADNAN AHMED[5]

[1]*Department of Environmental Sciences, University of Peshawar, Pakistan*
[2]*Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Dir Upper, Pakistan*
[3]*National Center of Excellence in Geology, University of Peshawar, Pakistan*
[4]*Department of Environmental Science, International Islamic University, Islamabad, Pakistan*
[5]*Department of Forestry, Shaheed Benazir Bhutto University Sheringal, Dir Upper, Pakistan*
[*]*Corresponding author's email: jumamohammad27@yahoo.com; sardar.khan2008@yahoo.com*
Tel: 0092 944 885832; Fax 0092 944 885805

Abstract

Dir-Kohistan (Pakistan) is a famous habitat of morel mushroom species and particularly rich with yellow morel (*Morchella esculenta*). This study was conducted to investigate both essential and non-essential metal concentrations in *M. esculenta* and soil collected from the study area. Results indicated that *M. esculenta* accumulated enough amounts of essential metals (Na, K, Ca, Fe and Zn). The nonessential metal concentrations in *M. esculenta* were below the maximum permissible limits set by WHO/FAO. Low concentrations of nonessential/toxic metals in mushroom could be linked with low human activities such as less application of agrochemicals and no mining activities in the study area. The daily intake rate (DIR) of *M. esculenta* showed that toxic metals were not a serious threat to the human health due to less edible uses and lower concentrations of toxic heavy metals. Statistical analyses, like inter-elemental coefficient, revealed a positive correlation between the selected metals, while weak linear correlations were observed between the essential and toxic metals in *M. esculenta* and associated soil.

Key words: Mushroom, *M. esculenta,* Essential metals, Toxic metals, Bioaccumulation, H

Introduction

Morchella is a true or sponge morel which is a genus of edible mushrooms. Anatomically, it is closely related to simpler cup-o-fungi. *Morchella esculenta* (yellow morel), *M. conica* (black morel) and *M. deliciosa* (white morel) are the common types of *Morchella*. The first two species grow in spring season (March-May), while the latter one grows in the monsoon season (July-August). Morchella has honey-comb like shape and about 3-11 cm in height (Fig. 1). Morels are usually found in temperate forest zone (1500-3000 m elevation) and are having strong mycorrhoeizal (Fungi+root) association with plants like strawberry and ferns (Raja 1992) and elm tree destroyed by fire or disease (McLain *et al*., 2005). These mushrooms have three different types of fruiting patterns such as i) regularly, in a specific locality, ii) due to the death of an ectomycorrhizal associate, and iii) after a big disturbance such as forest fire or insect epidemic (Pilz *et al*., 2007). Morels are cold tolerant and have been found to give fruits at temperature less than 5.6˚C (Emery & Barron 2010).

Morels have many nutrients including carbohydrates, proteins, vitamins, minerals such as essential and non-essential or toxic metals (Fang *et al*., 2014). These are generally used for curing wounds, skin diseases, epilepsy, rheumatoid arthritis, heart ailments, cholera, irregular fevers, dysentery, diaphoretic, diarrhea, anesthesia, cold, liver disease, gall bladder infection and are also very effective in curing asthma, tumor, cholesterol, stress, diabetes and insomnia diseases (Bahl 1983). That is the reason that mushrooms are considered as a good source of diet and medicine for human beings. According to Yang *et al,* (1993) drugs containing morels are used for the treatment of various kinds of diseases in China since long ago.

Wild mushrooms have the ability to accumulate both essential and non essential metals such as sodium (Na), potassium (K), copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As) (Kalac 2001; Fang *et al*., 2014) (Fig. 1). The accumulation of these metals is generally species-dependent in mushrooms (Li *et al*., 2011), however, the metal uptake is mainly dependent on environmental and genetic factors (Jarzynska & Falandysz, 2012b; Falandysz & Borovicka, 2013).

Metal concentrations in human diets were classified into essential (Na, K, Cu, Zn, Ca, Mg, Fe and Mn) and non-essential or toxic metals (Hg, Cd, Pb, Cr and As) (Khan *et al*., 2012, 2013; Shah *et al*., 2012; Yousuf *et al*., 2015). Low intake of essential metals produces deficiency, while higher consumption may cause toxicity. However, non-essential metals are lethal and toxic even at low concentrations to human and environment (Muhammad *et al.,* 2013; Shah *et al.,* 2013; Khan *et al.,* 2015). Non-essential metals are ranked among the most hazardous toxic substances owing to their persistence in the environment and absorption in food chain (Muhammad *et al*., 2011). Toxic effects of metals include vomiting, diarrhea, headache, irritability, hypertension, heart, lung, kidney, liver and intellectual problems and cancer (Shah *et al.,* 2012, 2013).

Several studies reported different opinions regarding fungi (mushrooms) as accumulative bio-indicators of soil pollution (Cocchi *et al*., 2006; Kalac 2010). Mushrooms that are grown in natural habitats, geochemically anomalous areas and anthropogenically polluted soils can uptake metals and concentrate them in the edible parts; thus, these toxic metals represent serious threats to the human beings (Aloupi *et al*., 2012; Falandysz *et al*., 2012; Fang *et al.,* 2014; Wang *et al*., 2014). Therefore, this study was aimed to investigate the essential and toxic metal concentrations and their enrichment in the soil and *M. esculenta* (mushroom) of the northern rural areas of Pakistan.

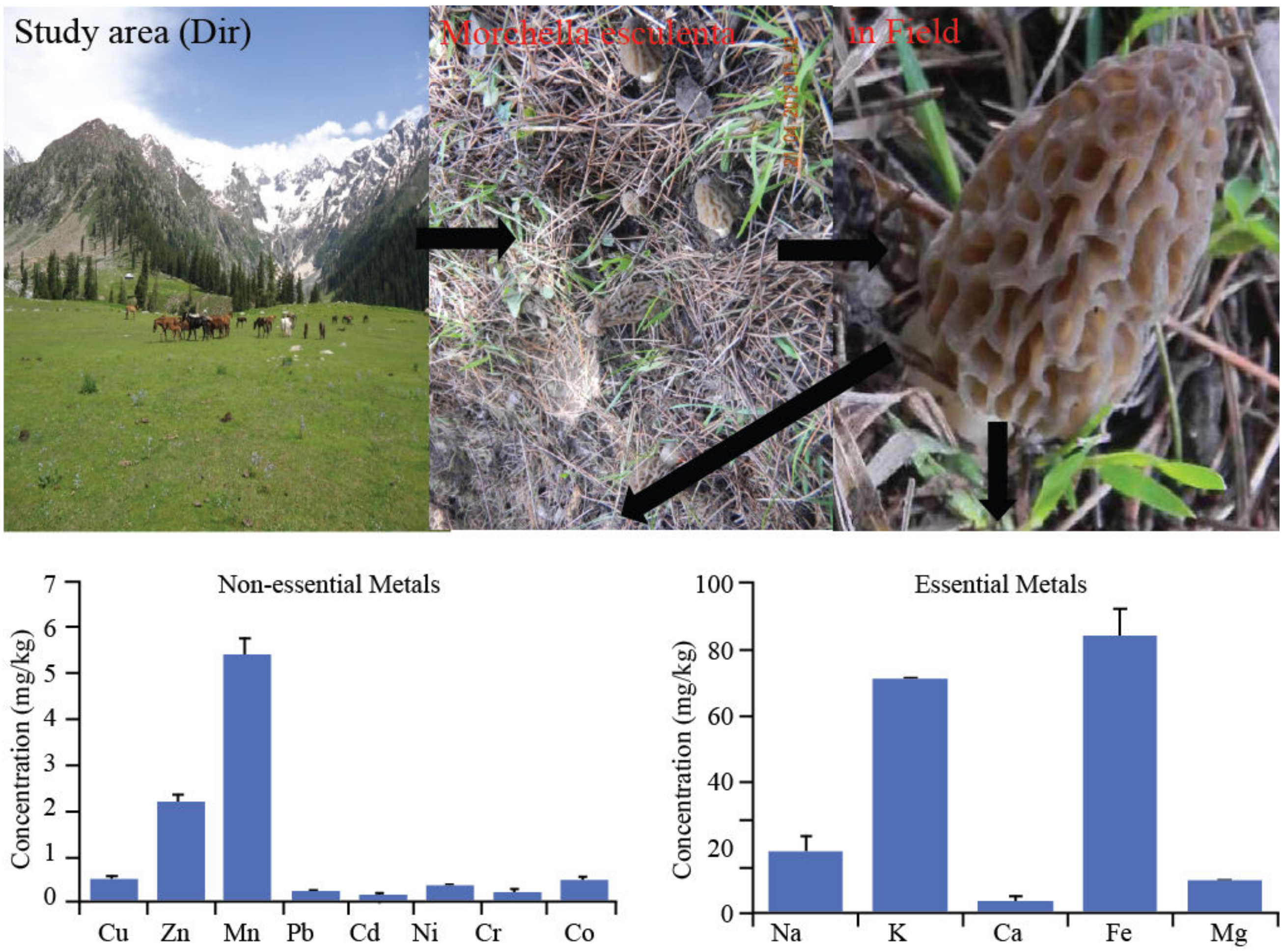

Fig. 1. The habitat of *M. esculenta* and essential and non-essential metals

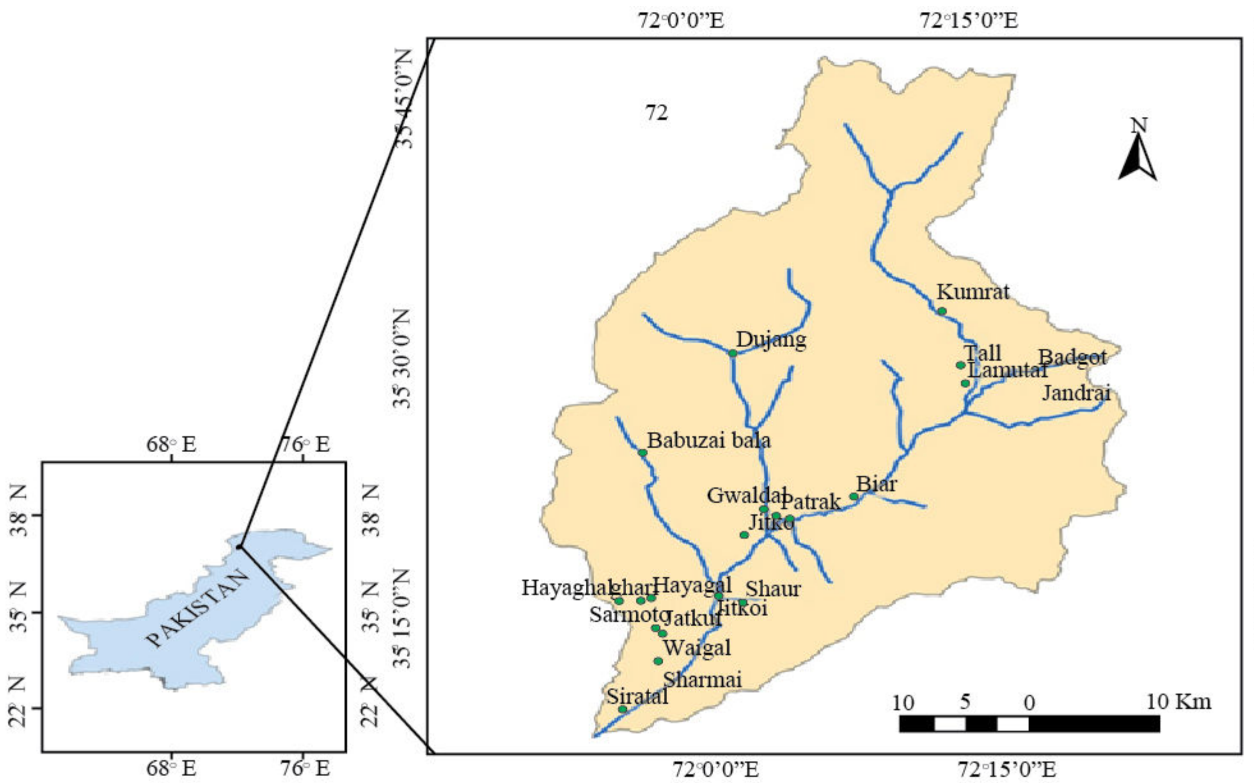

Fig. 2. Location map of the study area (Dir-Kohistan, Pakistan)

Methods and materials

Sampling and analyses: The study area is comprised of Jandrai & Kumrat valleys of Dir District, which is located at a distance of 365 km from the Peshawar City towards north of Pakistan. April to June is the favorable season for the collection of *Marchella* species in Dir-Kohistan. *Marchella* and associated soil samples were collected in triplicates from twelve sampling sites in April, 2012 (Fig. 2). All samples were packed in polythene bags, properly labeled and transported to the Geochemistry Laboratory of the National Center of Excellence in Geology (NCEG) and Centralized Resources Laboratory (CRL), University of Peshawar, for further processing and analyses.

Soil samples were air dried and sieved through 2 mm mesh sieve. Soil pH and electrical conductivity (EC) were determined according to the procedure adopted form Muhammad *et al.,* (2011, 2013). Nitrate (NO_3), sulphate (SO_4) and phosphate (PO_4) were determined in soil using the HACH Spectrophotometer (DR 2800). For metal extraction, the digestion tubes were properly acid washed and dried. Dry powdered sample of soil (0.5g) and *Morchella* (2 g) were taken in digestion tubes and 15ml of HNO_3, $HClO_4$ and H_2SO_4 (5:1:1) were added to each tube and were kept overnight. The tubes were then placed at 80˚C in the digestion block for about one h and then raised the temperature slowly increased up to 120-130˚C for *Morchella* and 160-180˚C for soil samples. When digestion was completed, the solutions were cooled, filtered and diluted to 50ml with double deionized water. In the filtrates, the selected essentials (Na, K, Ca, Fe and Mg) and toxic (Pb, Cd, Cu, Ni, Cr, Mn, Co and Zn) metals were determined using atomic absorption spectrometer (Analyst 700 of Perkin Elmer) in the CRL and Geochemistry Laboratory of the NCEG, , University of Peshawar.

Bioaccumulation factor (BF): Bioaccumulation factor of heavy metals in mushroom fruit body was calculated as:

$$BF = \frac{C_{fruit}}{C_{soil}}$$

where, C represents the metal concentrations in fruit and soil.

Risk assessment: Risk assessment in the study area was calculated through daily intake rate (DIR) and hazard quotient (HQ).

Daily intake rate (DIR): The metals' DIR values through ingestion of *M. esculenta* were calculated by the equation of Orisakwe *et al*., (2012);

$$DIR = \frac{C_{metal} \times D_{food\ intake}}{B_{average\ weight}}$$

where C represents concentration of metal, D is the daily intake of morels (0.00384 Kg, the ingestion rate is very less due to its high economic value) and B is the average body weight (60 kg).

Hazard quotient (HQ): Hazard quotient or chronic risk assessment in the study area was calculated according to the equation adopted from Khan *et al*., (2008).

$$HQ = \frac{DIR}{RfD}$$

where RfD represent the reference dose of metals. Oral reference dose for Cd, Cr, Ni, Pb, Zn, Cu and Fe are 1.0E-03, 1.5E-0, 2.0E-2, 3.5E-3, 3.0E-1, 4.0E-2 and 3.0E-1mg/kg/day, respectively (Khan *et al*., 2008).

Statistical analyses: Results were expressed as mean and standard deviation values. Data were analyzed by using MS Excel and inter elemental correlation using Pearson's correlation coefficients by SPSS software version 21.

Results and Discussion

Heavy metals in *M. esculenta*: Table 1 summarizes the concentrations and bioaccumulation factor (BF) of essential and toxic metals in *M. esculenta* of the study area. Among essential and toxic metals, the uppermost (92.74 mg/kg) concentrations were observed in morels for Fe and the lowermost (0.12 mg/kg) for Cd (Table 1). Morels showed slightly higher concentration of essential metals (Ca, K, Fe, Na and Mg). However, toxic metals revealed lower concentrations and were found within the safe level of WHO/FAO (Anon., 1999) (Table 1).The essential metals like Na, K, Ca, Fe have their nutritional and clinical significance, while the toxic metals like, Pb, Cd and Cr have toxic effects on the human health (Fang *et al.,* 2014; Wang *et al*., 2014). Metal concentrations of morals in the study area were found much lower than those reported by Genccelep *et al.* (2009) in Turkey and Michelot *et al.* (1998) in France. These lower metal concentrations in morels may be attributed to soil leaching due to heavy rain and snowfall. Secondly, the study area is thick forest zone where no anthropogenic activities are carried out. However, the recently started agricultural activities in Kumrat valley may have contaminated the soil due to applications of fertilizers and pesticides. Urban soil and water receive huge pollution loads of metals from different anthropogenic sources; especially agriculture, municipal waste, industrial effluents, garages and automobile emissions (Yusuf *et al.,* 2003).Therefore, the morel grown in urban soil may have the chances of accumulating more toxic metals. The ability of mushroom to act as bio-sorbents of toxic metals has been widely assessed (Murugesan *et al*., 2006). The uptake level of toxic metals in morel is different from the plant in many respects (Falandysz *et al*., 2003).

Metals intake of *M. esculenta*: According to the questionnaires survey (n=80) conducted in the study area, the average body weight of individual was reported as 60 kg. Similarly, the average collection of *M. esculenta* was 20 kg/year out of which only 7% (1.4 kg/year) is used for food (medicinal and delicious food) purposes, while remaining exported to other countries due high economic values.

The risk assessment (DIR and HQ) of *M. esculenta* are summarized in the Table 1. The highest DIR values (4.52E-03 mg/kg-day) were found for K, while the lowest (1.06E-05mg/kg-day) for Cd (Table 1). These result revealed lower DIR values for essential and toxic metal in the study area. Similarly, the chronic risk or HQ values were the highest (1.06E-02) for Cd and the lowest (9.60E-06) for Cr. This higher HQ value of Cd may be caused toxicity but HQ values for other toxic metals were found< 1 in the study area. Therefore, toxic metal concentrations in morel may not pose chronic risk to the local population.

Table 1. Essential and non-essential or toxic metals (mg/kg) concentrations, bioaccumulation factors and risk assessment of *M. aesculenta* in the study area.

Metals	Range	Mean±SD	BF[a]	Safe limits	Turkey[b]	France[c]	DIR mg/kg-day	HQ
Na	14.43-31.12	19.35±4.56	0.7	NA[d]	180	5740	8.72E-04	NA
K	70.76-71.58	71.12±0.24	1.01	NA	2350	586	4.52E-03	NA
Ca	2.38-7.16	4.18±1.63	1.21	NA	850	1040	3.24E-04	NA
Fe	60.35-92.74	84.08±8.19	0.44	NA	195	NA	2.38E-03	8.19E+04
Mg	9.95-10.46	10.27±0.16	0.9	NA	1810	NA	6.57E-04	NA
Cu	0.35-0.63	0.51±0.07	0.8	40	73.4	46.4	3.24E-05	8.10E-04
Zn	1.83-2.45	2.20±0.17	1.5	60	133	208	1.41E-04	4.70E-04
Mn	4.80-6.00	5.39±0.36	0.51	500	16.9	24	3.45E-04	NA
Pb	0.21-0.31	0.25±0.03	0.61	0.3	NA	44.2	1.57E-05	4.50E-03
Cd	0.12-0.20	0.17±0.03	0.84	0.2	NA	3.6	1.06E-05	1.06E-02
Ni	0.31-0.43	0.37±0.03	0.6	67	NA	15.4	2.37E-05	1.19E-03
Cr	0.16-0.30	0.23±0.05	0.3	2.3	NA	5.98	1.44E-05	9.60E-06
Co	0.39-0.65	0.49±0.05	0.69	NA	NA	3.2	3.10E-05	NA

[a]Bioaccumulation factor; [b]Genccelep *et al*., 2009; [c]Michelot *et al*., 1998; [d] Not allotted

Table 2. Inter-correlation of metals in the study area.

a. Soil

Metals	Ca	Cu	Zn	Mn	Pb	Cd	Ni	Fe	Cr	Na	Mg	K	Co
Ca	1.000	**0.727**	**0.525**	0.342	0.097	0.086	-0.504	-0.062	-0.188	0.024	0.033	0.493	-0.480
Cu		1.000	0.278	0.513	0.032	0.227	-0.108	0.046	-0.108	0.154	0.239	0.465	-0.410
Zn			1.000	0.417	0.091	-0.065	-0.142	0.260	0.421	0.022	0.153	0.055	0.121
Mn				1.000	-0.001	-0.271	0.424	-0.101	0.057	-0.457	0.312	0.341	0.243
Pb					1.000	0.436	0.213	**0.811**	0.405	0.401	**0.690**	-0.480	0.478
Cd						1.000	-0.209	0.289	0.030	0.354	0.152	-0.180	-0.270
Ni							1.000	0.356	0.464	-0.116	0.268	-0.410	**0.529**
Fe								1.000	**0.541**	**0.531**	0.461	-0.730	0.261
Cr	`								1.000	-0.026	-0.056	-0.710	0.344
Na										1.000	0.146	-0.210	-0.390
Mg											1.000	-0.050	**0.554**
K												1.000	-0.360
Co													1.000

b. *M. esculenta*

Metals	Ca	Cu	Zn	Mn	Pb	Cd	Ni	Fe	Cr	Na	Mg	K	Co
Ca	1.000	0.162	-0.326	-0.193	-0.403	0.090	-0.430	-0.043	-0.467	-0.032	-0.510	-0.165	0.013
Cu		1.000	0.350	0.240	-0.219	-0.582	-0.768	-0.370	-0.382	-0.328	-0.041	-0.346	0.272
Zn			1.000	0.438	0.315	0.033	0.047	-0.084	0.352	0.304	0.277	0.344	0.241
Mn				1.000	0.027	-0.237	0.121	0.389	0.425	0.156	0.447	0.005	0.339
Pb					1.000	0.447	0.445	-0.473	**0.579**	0.230	0.306	0.496	0.378
Cd						1.000	**0.721**	0.039	**0.554**	**0.593**	-0.174	0.355	0.070
Ni							1.000	0.342	**0.810**	**0.507**	0.132	0.419	0.100
Fe								1.000	0.294	0.327	0.019	-0.174	-0.443
Cr									1.000	0.377	0.321	0.188	0.342
Na										1.000	-0.225	**0.518**	-0.247
Mg											1.000	-0.145	0.026
K												1.000	0.055
Co													1.000

Bioaccumulation factor: Table 1 also summarizes the BF values of essential and toxic metals in morels of the study area. The highest BF value (1.50) was found for Zn, while the lowest (0.30) was found for Cd (Table 1).The BF values depend on the available fraction of metal and organic matter contents in the soil (Khan *et al*., 2006).Among essential and toxic metals, the BF values were found > 1 for K, Ca and Zn. These higher BF values of essential metals could fulfill the human body requirement and avoid the deficiency effects. However, the BF values of the toxic metals like Pb, Cd, Cu and Ni were found <1 suggesting that *M. esculenta* is not hyper accumulator of these metals.

Statistical analyses: Table 2 summarizes the statistical analyses such as inter-elemental correlation of the essential and toxic metals in morels and associated soil. However, in the study area, some metal pairs like Ca-Cu (r = 0.727), Cu-Mn (r = 0.513), Fe-Cr (r = 0.541), Fe-Na (r = 0.531), Pb-Mg (r = 0.690), showed positive correlation in soil (Table 2a). Similarly, in morels metal pairs like Pb-Cr (r = 0.579), Ni-Cr (r = 0.810) and Cd-Na (r = 0.593) showed positive correlation (Table 2b). These correlations revealed that essential and toxic metals have geogenic sources of contamination. Correlation between the metals in the morels and soil (Fig. 3), revealed that this relationship was not strongly significant, except for Na (r=0.516) and Mg (r=0.802).These weaker correlations of essential and toxic metals could be attributed to the morels physiologies and their variable uptake rate and soil properties and greater variation of these metals concentrations in the soil of the area (Khan *et al*., 2008; Angeles *et al.,* 2009; Muhammad *et al.,* 2013; Shah *et al*., 2013).

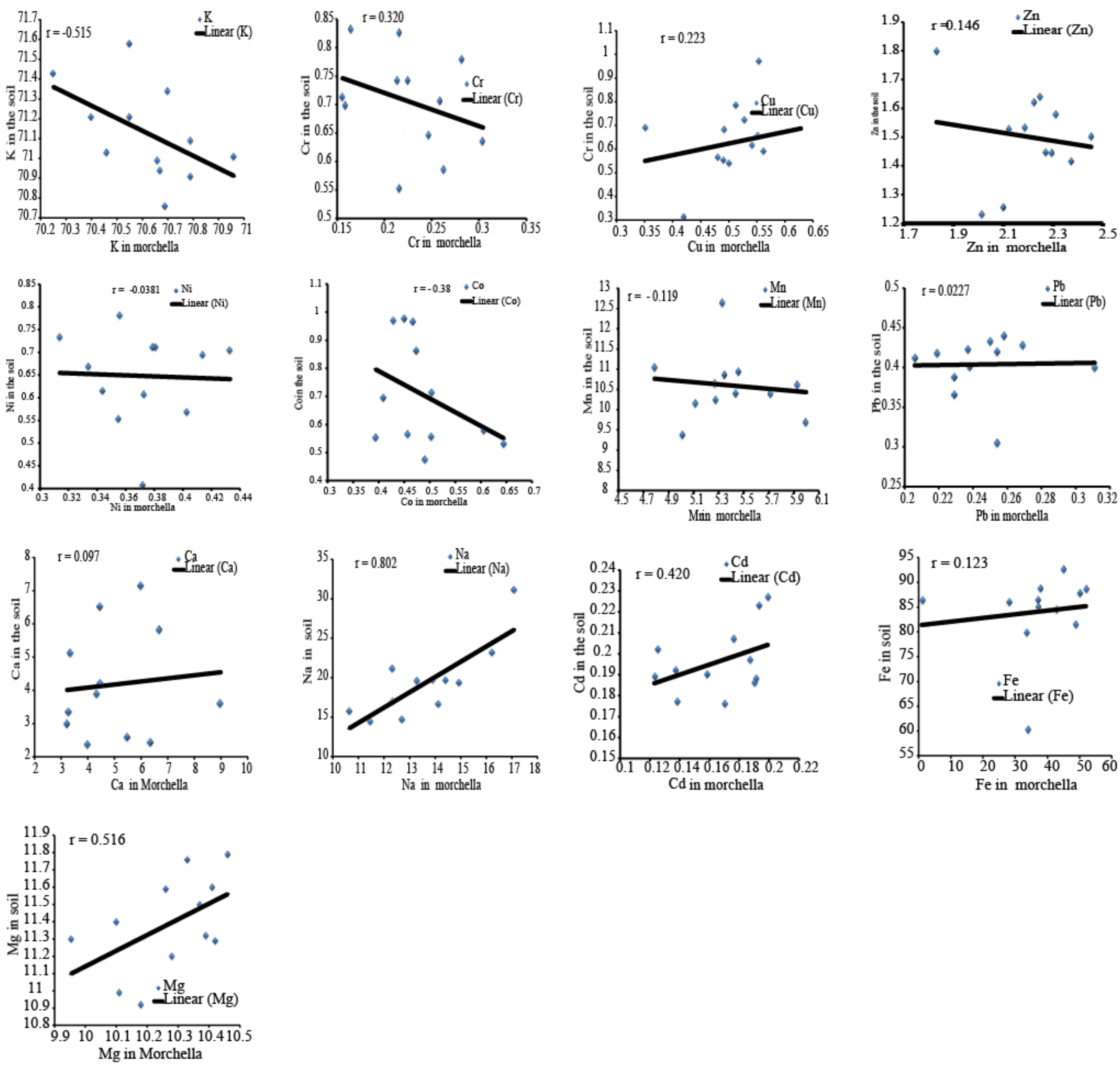

Fig. 3. Correlation between essential and non-essential metals concentrations in morels and soil of the study area.

Conclusions

Results of the study area showed that the selected metals concentrations were found below the safe limits of WHO/FAO set for edible mushrooms and for food stuff. The lower concentrations of essential and toxic metals in morels resulted in low DIR and HQ values. This could be attributed due to lack of anthropogenic input like mining and industry and low scale agricultural activities. BF value was found > 1 for essential metals and <1 for most of the toxic metals. Weaker inter-elemental correlation was observed in both morels and soil. Statistical analyses revealed that morel correlation with associated soil was found significant only for Na (r=0.802). This study concluded that the essential and toxic metals concentrations in morels were within the safe limits for human intake and poses no potential health risk. Therefore, this study strongly recommends the use of morels as a source of human diet in the study area to fulfill the essential metal requirements.

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