Mineral and heavy metals content in tilapia fish (Oreochromis niloticus) collected from the River Nile in Damietta governorate, Egypt and evaluation of health risk from tilapia consumption

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Abstract

This study was conducted to determine heavy metals and trace elements content in tilapia fish collected from three sources in Damietta governorate, Egypt and to evaluate the human health risk due to tilapia consumption. Tilapia samples were collected from two locations in the River Nile stream, two fish farms and two sluiceways. Health risk assessment was evaluated based on the consumption habits of adult human. The results revealed that all samples vary in elements concentrations. The calculation of human health risk revealed that the consumption of tilapia in the three tested area does not pose any health risk except for Selenium. It could be concluded that consumption of such fish may be a risk for consumers who eat fish more than one time per week. Consequently, precautions should be taken and warning against eating tilapia fish caught from these regions should be announced.

Keywords: Tilapia fish; Heavy metals; trace elements; health risk; river Nile; Egypt

Introduction

Fish is an essential source as nourishment protein and the expansion of population increase fish request (Qiu et al., 2011). Nile tilapia (Oreochromis niloticus) is refined generally in sub-tropical and tropical locales of the world and constitutes one of the largest groups of cultivated fish following salmonids and carps (Justino et al., 2016). The ideal fish health is very much accomplished by raising fish in a perfect situation free from various sources of pollutants and infectious agents, with good nutrition and minimum of stress. Recently, an overall consideration has been paid to the issues of the pollution of environment by various metals (Qadir and Malik, 2011; Aktar et al., 2011; Maceda-Veiga et al., 2012). It is surely understood that metals are of an imperative concern in light of their poisonous quality and their capacity to bioaccumulation in the aquatic ecosystems and their ecological persistency (Mohammadi et al., 2011). Biswas et al. (2011) reported that a few metals are conceivably harmful (arsenic, cadmium, mercury, and so forth) and numerous are essential (copper, zinc, iron, manganese). These essential metals can also induce toxic effects if the metal intake is excessively elevated (Tekin-öz, 2008; Félix et al., 2013). Previous studies revealed that fish is extremely delicate to poisonous substances present in water for all water
inhabitants (Shefer et al., 2015). Additionally, fish is the principle aquatic food chain which may concentrate a lot of metals from their water surroundings. Besides, fish is considered the most indicative factor in freshwater systems for the estimation of metal contamination and potential danger for human consumption when metals enter the human body and accumulate in the different tissues to pose chronic toxicity (Aktar et al., 2011; Freije, 2015). Therefore, the determination of metals content in fish is critical from the human wellbeing perspective (Biswas et al., 2011; Aung and Chang, 2014). The objectives of this work were to assay different elements and heavy metals concentrations in fish flesh collected from different locations of the Nile River in Damietta city (Damietta branch of the Nile River) in the North Delta of Egypt and to evaluate the human health risk from tilapia consumption.

Materials and methods

Sampling sites

The samples of Nile tilapia (Oreochromis niloticus) weighed 150 g each were collected from 6 locations of the River Nile in Damietta city (Figure 1). Two were locations within the River Nile stream, the first at the South of Damietta city (L1) and the second at the North of Damietta city (L2). Two fish farms in the River Nile, the first near by Damietta dam (L3) and the second near by wastewater and water station (L4). The other two locations were in sluiceway, the first at Shata village (L5) and the second at Shaaraa village (L6). Sixty fish samples from each location were collected using fishing nets.

Samples preparation and analysis

The flesh from each fish was dissected, washed with distilled water and weighed. After dissection, all samples were divided randomly into three pooled samples (each pooled sample contained twenty fleshes of tilapia fish). The pooled samples were dried at 105 °C and then uniformly ground in to powder and stored at -20 °C in order to determine metal concentrations. The following metals were determined: Iodine (I) content was determined according to the method described by Leufroy et al. (2015). Selenium (Se) and zinc content were analyzed according to Olmedo et al. (2013). The analysis of the zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were carried out using atomic absorptions spectrophotometry (AAS III, Carl Zeiss Jena, Germany). All elements analyses were carried out in the Institute of Nutrition and Environment, Friedrisch-Schiller Uni. Jena, Germany. All of the glassware and plastics were soaked over-night in 10% (v/v) nitric acid, rinsed with distilled and deionized water and were dried before being used.

Quality assurance

The accuracy and precision of our results were checked by analyzing standard reference material obtained from the National Institute of Standards and Technology (NIST SRM 1577b, Gaithersburg, MD, USA). Replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 96% and 105%. All metal concentrations were quoted as mg/kg dry weight.

Estimation of health risk assessment

Target Hazard Quotient (THQ)

To estimate the risk of heavy metals, the
following equation was used:

\[ \text{THQ} = \left( \frac{EF \times ED \times FI \times C}{\text{RFD} \times \text{WAB} \times \text{TA}} \right) \times 10^{-3} \]

Where:

- EF is exposure frequency (52 days/year for people assuming to eat tilapia fish once a week);
- ED is the exposure duration (average people life time, 70 years);
- FI is the amount of fish ingestion (fish: 250g day/person) (FAO, 2009);
- C is the metal concentration in the edible fish part (mg/g);
- RFD is the oral reference dose (US-EPA, 2000);
- WAB is the average body weight (70 kg);
- TA is the average exposure time for non-carcinogens (365 days/year x ED).

Calculation of the allowable daily consumption \( (\text{CR}_{\text{lim}}) \)

Assumption that no other sources of heavy metals were found in diets, \( \text{CR}_{\text{lim}} \) was calculated using the following equation (kg fish/day):

\[ \text{CR}_{\text{lim}} = \frac{\text{RfD} \times \text{BW}}{\text{Cm}} \]

Where

- \( \text{CR}_{\text{lim}} \): maximum safe daily consumption rate of tilapia (kg/day);
- RfD: reference dose for each heavy metals (mg kg/day);
- BW: average consumer body weight (kg);
- Cm: measured concentration of chemical in the edible portion of fish (mg/kg). The RfD is determined by the U.S. EPA and is an estimate of the daily intake of a contaminant over a life time that would not be expected to cause adverse health effects (US-EPA, 2000).

Calculation of maximum allowable tilapia consumption \( (\text{CRmm}) \)

\( \text{CRmm} \) was calculated by the following equation

\[ \text{CRmm} = \frac{(\text{CR*}_{\text{lim}} \times \text{Tap})}{\text{MS}} \]

Where

- \( \text{CRmm} \): maximum allowable tilapia consumption rate (meals/month), \( \text{CR*}_{\text{lim}} \): maximum weekly consumption rate of tilapia fish (kg/week), \( \text{Tap} \) is the average time period in a month (4.3 week/month), \( \text{MS} \): meal size, 227g for adults (US-EPA, 2000).

Statistical analysis:

All data were subjected for statistical analysis using the General Linear Model Procedure of the Statistical Analysis System (SAS, 1994). Duncan’s multiple range test (Duncan, 1955) was performed when the differences were significant. Mean values were considered significantly different at \( P<0.05 \). The data were expressed as mean values ± standard error.

Results

The results of trace elements content in the fish fleshes are presented in Table (1). These results indicated that iodine content was significantly higher in the fish farm in the River Nile located near by Damietta dam (L3). However, no significant differences in iodine content in fish fleshes collected from the other locations although the samples collected from the first location in the sluiceway (L5) showed insignificant increase compared to the other locations. On the other hand, the overall mean of iodine concentration revealed that the samples collected from the two fish farms was significantly higher than that for fish samples collected from the other two locations (Fig. 2a). The concentration of selenium was significantly higher in fish collected from L1 compared to its concentration in fish samples collected from the other locations. Moreover, the concentration of selenium in fish samples collected from L2, L4 and L6 was higher than its concentration in fish samples collected from L3 and L5. The overall mean for selenium concentration in fish samples collected from the River Nile (L1 and L2) was significantly higher compared to the concentration of selenium in fish samples collected from the other locations (Fig. 2b). The fish samples collected from L2 and L6 showed the highest zinc concentration compared to the other samples. While the lowest zinc concentration was found in fish samples collected from L3. The overall mean for zinc concentration in fish collected from the fish farms showed a significant decrease compared to the overall mean for zinc concentration in fish samples collected from the River Nile locations or the sluiceway locations (Fig 2c). The current data also revealed that no significant differences were found in iron content between the samples.
Table 1 Trace elements concentrations in fish flesh collected from different locations of River Nile in Damietta city (means ± SE).

| Location | Element   | River Nile stream | Fish farm | Sluiceway | Recorded levels (Ref) |
|----------|-----------|-------------------|----------|-----------|-----------------------|
|          | L1        | L2                | L3       | L4        | L5        | L6               |
| Iodine   | (µg/kg DM)| ± 154.15<sup>a</sup> | ± 157.62<sup>a</sup> | ± 705.81<sup>b</sup> | ± 137.99<sup>a</sup> | ± 19.63<sup>a</sup> | ± 265.87<sup>a</sup> | 116 (Menon and Skeaff, 2016) |
| Selenium | (µg/kg DM)| ± 0.28<sup>b</sup> | ± 0.01<sup>c</sup> | ± 0.02<sup>c</sup> | ± 0.05<sup>b</sup> | ± 0.02<sup>c</sup> | ± 0.01<sup>b</sup> | 2.70 (Ling et al. 2013) |
| Zinc     | (mg/kg DM)| ± 7.37<sup>a</sup> | ± 4.73<sup>b</sup> | ± 0.28<sup>c</sup> | ± 12.81<sup>d</sup> | ± 1.95<sup>a</sup> | ± 1.19<sup>b</sup> | 61.4 (Ling et al. 2013) |
| Iron     | (mg/kg DM)| ± 93.71<sup>a</sup> | ± 36.91<sup>b</sup> | ± 62.53<sup>c</sup> | ± 331.15<sup>b</sup> | ± 99.71<sup>a</sup> | ± 349.18<sup>b</sup> | 100.73 |
| Copper   | (mg/kg DM)| ± 5.45<sup>a</sup> | ± 0.80<sup>b</sup> | ± 0.35<sup>b</sup> | ± 0.12<sup>c</sup> | ± 4.25<sup>d</sup> | ± 0.31<sup>c</sup> | ± 0.21<sup>b</sup> | 3.17 (Koch et al. 2016) |
| Manganese| (mg/kg DM)| ± 6.05<sup>a</sup> | ± 2.64<sup>c</sup> | ± 23.63<sup>c</sup> | ± 54.15<sup>c</sup> | ± 8.96<sup>a</sup> | ± 13.32<sup>d</sup> | 13.3 (Ling et al, 2013) |

Within the same row, means superscript with different letters (a, b, c) are significantly different (P < 0.05). Within the same row, means superscript with different letters (A, B) are significantly different (P < 0.05).

Fig. 2. Trace elements concentration in the three studied areas collected from L1, L3 and 5 however; those collected from 2, 4 and L6 showed a significant higher iron concentration than the other studied locations. The overall mean of iron concentration revealed that samples collected from the two fish farms locations had a lower iron content compared to the samples collected from the other locations (Fig. 2d). The concentration of copper in the fish samples was varied between all the locations except those collected from L2 and L6. The higher concentration in copper was found in the samples collected from L4 and the lowest concentration was recorded in the samples collected from L3 and L5. The overall mean for copper concentration revealed that the higher concentration was found in the samples collected from the fish farms followed by those collected from the River Nile then the samples.
collected from the sluiceway (Fig. 2e). The data presented in Table (1) also revealed insignificant differences in manganese concentrations between the fish samples collected from L1 and L5 or those collected from L2 and L3. However, the samples collected from L4 contained the highest manganese concentration. On the other hand, the overall mean of manganese concentration indicated that the samples collected from the fish farms contain the highest concentration of manganese compared to the other collected samples (Fig. 2f).

The results of microelements and heavy metals concentrations in the samples of fish flesh collected from the different locations are presented in Table (2). The concentration of calcium was significantly higher in the samples collected from L2, L4 and L6 than those collected from L1, L3 and L5 although the overall mean of calcium concentration in all the tested samples revealed that the samples collected from the fish farms contain the lowest calcium concentration (Fig 3a). No significant differences were recorded in Magnesium concentration between the samples collected from L1, L3 and L5. However, the samples collected from L2 and L4 contained the highest magnesium concentration and the lowest concentration was recorded in the samples collected from L6. The overall mean of magnesium concentration in all the studied samples showed significant differences between the three main locations and the highest magnesium concentration was recorded in the samples collected from the fish farms (Fig 3b). Sodium concentration showed insignificant differences between the fish samples collected from L1, L3 and L5. However, the samples collected from L2 and L4 were the highest in sodium concentration followed by those collected from L6. On the other hand, the overall mean showed that no significant difference was observed in sodium concentration between the fish samples collected from the River Nile locations and the fish farm locations and the lowest sodium concentration was found in the fish samples collected from the arsenic concentration sluiceway locations (Fig. 3g). The current data revealed the higher concentration of mercury was found in the fish samples collected from L1 followed by those collected from L8 however; no significant differences were found between the samples collected from the other locations. The overall mean for mercury concentration indicated that the samples collected from the River Nile locations contained the highest concentrations followed by those collected from the sluiceway locations (Fig. 3h).

The human health risk assessment for the toxic heavy metals for the toxic heavy metals was estimated using three methods included the calculation of the target health quotient (THQ), the calculation of the allowable daily consumption (CR_{lim}) and maximum allowable tilapia consumption rate as meals/ month (CR_{mm}). The results revealed that no human health risk was found (Table 3) since THQ was less than 1 in all the studied area assumed that people consume 250
Table 2: Microelements and heavy metals concentrations in fish flesh collected from different locations of River Nile in Damietta city (means ± SE)

| Location   | Calcium | Magnesium | Sodium | Potassium | Lead | Cadmium | Arsenic | Mercury |
|------------|---------|-----------|--------|-----------|------|---------|---------|---------|
|            | (mg/kg DM) | (mg/kg DM) | (mg/kg DM) | (mg/kg DM) | (mg/kg DM) | (mg/kg DM) | (mg/kg DM) | (μg/kg DM) |
| River Nile stream | 49821 ± 4625<sup>a</sup> | 1261.1 ± 51.95<sup>a</sup> | 5.57 ± 0.33<sup>a</sup> | 9.65 ± 1.07<sup>a</sup> | 0.21 ± 0.06<sup>a</sup> | 0.01 ± 0.01<sup>a</sup> | 0.29 ± 0.10<sup>a</sup> | 93.60 ± 4.85<sup>a</sup> |
| Fish farm   | 69921 ± 354<sup>a</sup> | 1817.7 ± 141.81<sup>b</sup> | 7.46 ± 0.80<sup>b</sup> | 11.85 ± 0.33<sup>b</sup> | 1.42 ± 0.03<sup>b</sup> | 0.06 ± 0.03<sup>b</sup> | 0.36 ± 0.02<sup>b</sup> | 7.00 ± 1.50<sup>b</sup> |
| Sluiceway   | 41181 ± 1878<sup>a</sup> | 1247.9 ± 65.77<sup>b</sup> | 5.10 ± 0.33<sup>b</sup> | 11.62 ± 0.62<sup>b</sup> | 0.09 ± 0.02<sup>b</sup> | 0.01 ± 0.01<sup>b</sup> | 0.21 ± 0.01<sup>b</sup> | 9.33 ± 3.15<sup>b</sup> |
| L1          | 70288 ± 566<sup>a</sup> | 2008.9 ± 31.49<sup>b</sup> | 7.97 ± 0.51<sup>b</sup> | 11.56 ± 0.50<sup>b</sup> | 1.41 ± 0.02<sup>b</sup> | 0.04 ± 0.23<sup>c</sup> | 0.50 ± 0.22<sup>c</sup> | 9.47 ± 3.71<sup>b</sup> |
| L2          | 45162 ± 1062<sup>a</sup> | 1370.0 ± 28.72<sup>b</sup> | 5.84 ± 0.51<sup>b</sup> | 12.58 ± 0.12<sup>b</sup> | 0.49 ± 0.15<sup>c</sup> | 0.001 ± 0.00<sup>d</sup> | 0.22 ± 0.01<sup>d</sup> | 14.70 ± 3.52<sup>b</sup> |
| L3          | 71838 ± 753<sup>b</sup> | 1593.5 ± 12.29<sup>c</sup> | 6.14 ± 0.04<sup>c</sup> | 11.67 ± 0.23<sup>b</sup> | 1.32 ± 0.09<sup>c</sup> | 0.07 ± 0.03<sup>c</sup> | 0.16 ± 0.01<sup>e</sup> | 33.20 ± 7.16<sup>c</sup> |
| L4          | 4140 ± 433<sup>a</sup>  | 240<sup>a</sup>          | —       | —         | —    | —       | —       | 0.5      |

Within the same row, means superscript with different letters (a, b, c) are significantly different (P < 0.05). Within the same row, means superscript with different letters (A, B, C) are significantly different (P < 0.001).

Fig. 3. Microelements and heavy metals concentrations in the three studied areas.
Table 3 Health risk estimates for toxic heavy metals ingestion from tilapia fish collected from the three tested locations

| Location Element | RFD (Wu et al., 2016) | Conc. (µg/g) | HQ | CR$_{\text{lim}}$ | Conc. (µg/g) | HQ | CR$_{\text{lim}}$ | Conc. (µg/g) | HQ | CR$_{\text{lim}}$ |
|------------------|-----------------------|--------------|----|------------------|--------------|----|------------------|--------------|----|------------------|
| Selenium         | 0.005                 | 0.00001      | 0.000001 | 3500            | 66.3         | 0.0003 | 0.00007          | 1166.67      | 22.09 | 0.0001          |
| Zinc             | 0.3                   | 22.09        | 0.09   | 0.95            | 0.01         | 24.25 | 0.01             | 0.87         | 0.02  | 15.68           |
| Copper           | 0.04                  | 11.68        | 0.15   | 0.24            | 0.005        | 17.74 | 0.05             | 0.16         | 0.03  | 24              |
| Lead             | 0.002                 | 0.216        | 0.013  | 0.65            | 0.01         | 0.20  | 0.01             | 0.7          | 0.01  | 0.186           |
| Cadmium          | 0.001                 | 0.0079       | 0.0009 | 8.86            | 0.17         | 0.008 | 0.0009           | 4.38         | 0.08  | 0.011           |
| Arsenic          | 0.004                 | 0.046        | 0.001  | 6.09            | 0.12         | 0.08  | 0.002            | 3.06         | 0.06  | 0.088           |
| Mercury          | 0.0001                | 0.009        | 0.011  | 0.78            | 0.0001       | 0.0001 | 0.02             | 0.0003       | 0.0001 | 0.004           |

Discussion

In Egypt, the River Nile is affected by serious and broad human activities, a significant extent of heavy metals in the environment is of anthropogenic origin. However, rock weathering, atmospheric deposition and phosphate mineral sources are the natural inputs of heavy metals to aquatic environments (Callender, 2014). Despite topography, the concentration of heavy metals in fish species were fluctuated in various areas according to different anthropogenic activities, for example, the releases from electroplating and the textile factories, ship antifouling paints, agricultural runoff and vehicle emissions which are contribute to the heavy metal pollution in marine sediments (Frei et al., 2014). In the current study, the levels of various heavy metals and trace elements were determined in tilapia fish samples collected from different locations in the River Nile in Damietta governorate, Egypt. The results revealed that iodine concentration in fish samples was 1493, 3628 and 1905 µg/kg DM for the three tested locations (River Nile, fish farms and sluiceway respectively). According to the National Research Council of the US National Academy of Sciences in 1989, the recommended dose of iodine was 2 µg/kg/day for adolescents and adults (Menon and Skeaff, 2016). Consequently, the current study revealed that iodine content in fish is higher than the suggested dose set by WHO. The concentration of selenium reported in the current study were 1.06, 0.568 and 0.509 µg/kg DM for the samples collected from the three tested locations. These concentrations were blew the recommended levels set by WHO (1987) and Wu et al. (2016) who suggested a level of 20.4 µg/day for adult females and 27.3 µg/day for adult males. Zinc concentration recorded 96.78, 63.10 and 92.36 mg/kg DM for the three tested locations respectively. These concentrations were higher than those reported previously (Borrell et al., 2016) in fish samples collected from the Pearl River Estuary, China who recorded 8.78-86.3 mg/kg DM. However, in a recent study, Leung et al. (2014) reported that the level of Zn was ranged from 0.02 to 38.2 in Tilapia collected from the same River.

The mean concentration of Fe reported in the current study revealed a high concentration of this metal in all fish samples. The recorded values were 215.31, 196.84 and 224.44 for the three locations, respectively. These concentrations were significantly higher than those reported by Mansour and Sidky (2002) who recorded concentrations ranged from 9.35 to 24.6 mg/kg DM in tilapia collected from fish farms in Fayoum, Egypt. Copper concentration recorded 7.08, 9.66 and 4.89 mg/kg DM for the three tested locations, respectively. These levels were higher...
than those reported by Leung et al. (2014) who recorded levels of Cu ranged from 0.92 to 1.72 mg/kg wet weight in Tilapia collected from Pearl River Delta, China. The same authors reported that the level of Mn in fish samples ranged from 0.82 to 6.91 mg/kg wet weight which were lower than those reported in the current study and were found to be 16.40, 38.89 and 11.14 mg/kg DM for the three tested locations, respectively.

The determination of microelements concentrations in the fish flesh samples collected from the three locations revealed that the concentration of calcium recorded 59871, 55734 and 58500 mg/kg DM, these results were extremely higher than that suggested by Mansour and Sidky (2003) who recorded Ca level of in tilapia fish collected from lake Qarun or Rayan lake, Egypt. Moreover, these concentrations are higher than that recommended by the National Institute of Health (NIH, 2013) who set daily dose of 1000 mg for adult men and 1200 mg for adult women. In the current study, the recorded levels of Magnesium were 1539.4, 1628.4 and 1481.8 mg/kg DM which were extremely higher than those recorded by Leung et al. (2014) who found that Magnesium concentration ranged from 0.82 to 0.91 in fish samples collected from China.

The Priority List of Hazardous Substances set up by the Agency for Toxic Substances and Disease Registry (ATSDR, 2013) reported that the descending order of heavy metals threatening to human health were As > Pb > Cd > Ni > Zn > Cr > Cu > Mn. Taken together, except for selenium, cadmium and arsenic, the current results revealed that the recorded data were higher than the published data or that the recommended doses. Consequently, consumption of the fish collected from the Damietta prevalence may put the consumer under risk. The current data also revealed that the freshwater in the River Nile or the connected sluiceway is highly polluted. On the other hand, tilapia is well known to consume vast varieties of natural foods such as plankton, green leaves, benthic organisms, larval fish detritus, aquatic invertebrate and decomposing organic matter (Yang et al., 2016). Thus, tilapia may ingest a considerable amount of contaminated sediment, together with other food items, and hence, rather high metal concentrations were found in the muscle of tilapia (Nakayama et al., 2010).

The human health risk assessment for the toxic heavy metals reported in the current study was calculated based on the amount of fish consumption of 250 g suggested by FAO (2009) and exposure frequency of 52 days/ year with assumption that people eat tilapia once a week for their life time of average 70 years for a people with average body weight of 70 kg. The US-EPA (2000) set an oral reference dose (RFD) for different heavy metals (Table 3). In this concern, Khan et al. (2008) reported that a potential health risk occurs if HQ reached 41 for the studied metal. Consequently, the current study revealed that fish consumption does not pose any health risk for the studied heavy metals in the three tested area for people who consume tilapia one time per week since THQ does not exceed one. CR_{min} and CR_{max} for Selenium were high for the three areas which may induce health risk even for the people who consumed tilapia for one time every week. These results were accordance with the report of the US-EPA (2000) and Shahbazi et al. (2016) who suggested that the maximum daily consumption rates of fish would not be expected to cause any adverse health effects for human.

Conclusion

The present study revealed that the fish samples collected from the River Nile, the farms located in the River Nile and the branched sluiceway were contaminated by various metals. The most abundant metals were iodine and mercury. These results reflex the water and/or fish diet pollution. Although the calculation of human health risk revealed that the consumption of tilapia in the three tested area does not pose any health risk except for Selenium, consumption of such fish may be a risk for consumers who eat fish more than one time per week. Consequently, precautions should be taken and warning against eating tilapia fish caught from these regions should be announced. Moreover, the governmental and the public sectors should pay a great attention for the public health protection.

Conflicts of interest

The authors declare that there are no conflicts of interest.
Acknowledgments

This work was supported by National Research Center, Dokki, Cairo, Egypt project # S90402.

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