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# Seasonal Variability in Cadmium, Lead, Copper, Zinc and Iron Concentrations in the Three Major Fish Species, *Oreochromis niloticus*, *Lates niloticus* and *Rastrineobola argentea* in Winam Gulf, Lake Victoria: Impact of Wash-Off into the Lake

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**Abstract** Trace metals Cadmium (Cd), Lead (Pb), Copper (Cu), Zinc (Zn) and Iron (Fe) were analyzed in edible portions of three main finfish species namely *Lates niloticus*, *Oreochromis niloticus* and *Rastrineobola argentea* sampled from various beaches of Winam Gulf, Lake Victoria, Kenya, in order to determine any seasonal and site variations and the results showed significantly ($p < 0.05$) higher mean concentrations of Cd, Cu, Zn and Fe during the wet season compared to the dry season for all the three species indicating the impact of wash-off into the lake during the rainy periods. The overall mean concentrations of the heavy metals (in µg/g dry weight) in all combined samples ranged from 0.17–0.40 (Cd), 0.47–2.53 (Pb), 2.13–8.74 (Cu), 28.9–409.3 (Zn) and 31.4–208.1 (Fe), respectively. It was found that consumption of *Rastrineobola argentea* can be a significant source of heavy metals especially Zn, to humans, compared with *Lates niloticus* and *Oreochromis niloticus*, if only the muscle parts of the latter two are consumed.

**Keywords** Fish · Human-consumption · Heavy-metals · Lake Victoria

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Winam Gulf of Lake Victoria is surrounded by various anthropogenic sources of contaminants including human settlements, livestock, agricultural farming activities and industrial effluents. The inflow into the lake comes through rivers, streams and industrial discharges which are believed to be carrying varying loads of toxic chemicals, particularly through wash-off during the rainy seasons. Previous reports have indicated existence of various toxic pollutants including pesticide residues, nutrients and heavy metals in a number of rivers which discharge into the Gulf (Kengara et al. 2004; Omwoma et al. 2010; Ongeri et al. 2010). Large loads of floating organic matter can often be seen on the surface of the lake water, especially during the rainy seasons, with % organic carbon content in sediment increasing up to 7.6% (Ongeri et al. 2010). In general, both agricultural and urban soils contribute to high concentrations of heavy metals (Omwoma et al. 2010) and since Winam Gulf is vulnerable to high loads of eroded soil particles and other debris, these form significant sources of bound pollutants including heavy metals during rainfall storms. Ongeri et al. (2010) recently reported seasonal variation in bio-available heavy metal concentrations in sediment, with bio-available fractions, which represent recent anthropogenic inputs of these metals from upstream and within the lake shores, especially during rainfall, ranging from 55.8 to 73.3%.

The presence of toxic metals in the lake is of particular concern because of their environmental persistence, bioavailability and toxicity to aquatic organisms and ability to be incorporated into food chains (Mwamburi and Oloo 1997; Soltan et al. 2005). Past studies indicate that water and sediment samples from Winam Gulf of Lake Victoria have fairly high levels of some of the heavy metals (Mwamburi and Oloo 1997) which justify the need to investigate the potential human exposure through consumption of fish harvested from it. In 1994, the most important species of fish harvested in Lake Victoria, as percentages of the total masses of fish landed, were *Lates niloticus* (Nile Perch) (55%), *Oreochromis niloticus* (Tilapia) (5.5%) and *Rastrineobola argentea* (Silver Cyprinid or Omena) (36%) (DDP 1994). However, these outputs fluctuate, and, in 2006, *Lates niloticus* significantly declined from 55% to 24% in the year while *Rastrineobola argentea* increased from 36% to 54% (LVFO 2008). *R. argentea* species has a significantly high fat content as most fat, which is concentrated around its internal organs, is needed to cushion it from shock. It is a common diet locally and is eaten whole, without removing internal organs and the head. It is also sold in high quantities for making chicken feed. However the muscle flesh of Nile perch and Tilapia, obtained after removal of their scales, internal organs, bones and heads, are used for human consumption. Nile Perch and Tilapia from this lake are also important export products mainly in the European Union and are faced with strict adherence to residue limits. This study aimed at determining the concentrations of Cd, Pb, Zn, Cu and Fe in the three most prevalent species of finfish in Winam Gulf and establishing if there is any seasonal effect on the concentrations of these metals through increased wash-off during the rainfall. The study only focused on the edible parts of the fish i.e., muscle of Nile Perch and Tilapia and whole fish samples of Omena.

## Materials and Methods

The sampling sites were located at four major fish landing beaches, Port Victoria, Sio Port, Dunga and Hippo Point, on the shores of the lake and were selected on the basis of existing inflowing perennial rivers and catchment characteristics. Port Victoria and Sio Port fish landing beaches in Busia district are very close to where River Sio discharges into the lake bringing in wash-off from human settlements, agricultural activities and soil erosion. There are also sand mining activities on Sio river, just as it enters into the lake. Dunga and Hippo point fish landing beaches are located in Kisumu city and are both near the discharge point of River Nyamasaria which flows through the densely populated human settlements and carries municipal and industrial effluents in Kisumu city and agricultural effluent from the Nyando area located upstream.

Fish samples were collected from the fishermen as the boats landed on the beaches. Five samples of each species were taken from each site during two rainy seasons and two dry seasons, respectively, between May 2006 and February 2007. The samples were transported in an icebox to the laboratory for storage, processing and analysis in Maseno University. *Lates niloticus* and *Oreochromis niloticus* samples measured from 15 to 20 cm in length and *Rastrineobola argentea* samples each weighing 100 g, were collected in polyethylene bags. Four replicates of fish samples were analyzed while the fifth replicate was kept in the laboratory as a backup. The dry weight of each fish species was determined according to the procedure of Ongeri et al. (2010). The fat (oil) contents were determined by Soxhlet extraction with n-hexane for 8 h, followed by rotary evaporation to dryness to give the net mass, expressed as % fat. The procedure of Ongeri et al. (2010) for tissue metal determination was followed and the samples were analyzed in an atomic absorption spectrophotometer (AAS) at 228.8, 324.8, 213.8, 243.3 and 283.3 nm, respectively. Statistical analyses were performed with SPSS and MSTATC software packages. Statistically significant differences were expressed at $p < 0.05$.

## Results and Discussion

The % fat (oil) (mean ± standard deviation), based on dry weight basis, was $2.12 \pm 0.06$, $2.77 \pm 0.11$ and $11.44 \pm 1.24$ in *Lates niloticus, Oreochromis niloticus* and *Rastrineobola argentea,* respectively. *Rastrineobola argentea* recorded significantly ($p < 0.05$) higher % fat content despite its small size compared to the other two species. Statistical analysis by ANOVA found least square difference (LSD) value of 2.24 for variations among fish species and no significant site variation ($p < 0.05$). There was little difference between the % fat contents in *Lates niloticus* and *Oreochromis niloticus*. The higher % fat content in *Rastrineobola argentea* could attributed to the fact that the fish was analyzed whole; with all its internal organs intact. *Rastrineobola argentea* species has been reported to have high fat content concentrated around its internal organs to cushion it from shock. The head, skin and other internal organs of fish which were removed from the two *niloticus* species before analysis have been associated with high fat contents (USEPA 1997).

The mean wet weight (ww)/dry weight (dw) ratios for *Lates niloticus*, *Oreochromis niloticus* and *Rastrineobola argentea* were $4.58 \pm 0.23$, $4.70 \pm 0.03$ and $5.05 \pm 0.24$, respectively. The ratios had no significant site and species variations ($p < 0.05$). The differences in these ratios were attributed to differences in % fat content among the three fish species. Since fish has a negative relationship between fish body density and body fat content, the decrease in weight during drying is likely to follow the trend *Rastrineobola argentea* > *Oreochromis niloticus* > *Lates niloticus* and this is what was observed in this study. The ww/dw ratios were used to express concentrations of metals in µg/g on a dry weight basis for convenient comparison with the EU and FAO permissible limit levels in fish.

The method detection limits and % recoveries for Cd, Cu, Zn, Pb and Fe ranged from 0.07–0.14 µg/kg and 79–92% (dry weight). The mean site concentrations of Cd, Pb, Cu, Zn and Fe expressed in µg/g (dry weight) for both the wet and dry seasons are presented in Tables 1 and 2. Analysis of variance (ANOVA) recorded significant variations ($p < 0.05$) in the average site concentrations of all the five metals analyzed among the sampling sites for the three fish species. Site variations were also significantly different ($p < 0.05$) for all metals. The least square difference (LSD) values for variations in heavy metal concentrations ranged from 0.02 (Cd)–6.75 (Zn) among fish species, 0.02 (Cd)–5.76 (Zn) among sites and 0.05 (Cd)–16.27 (Zn) between seasons. Any variations in concentrations of heavy metals among the three species of fish could be attributed to the differences in species diversity, their mobility, habitats and different feeding habits as well as differences in the portions and organs analyzed. *Lates niloticus* obtains its diet mainly from invertebrates including crustaceans, young and small fish, and shifts from one prey item to another depending on availability (Ogari and Dadzie 1988). In the Winam Gulf their prey diet has changed from the *haplochromine* to *caridina nilotica* and *Lates niloticus* juveniles (Ogari and Dadzie 1988). *Oreochromis niloticus* originally known to be herbivorous, feeds on algae (mainly), insect parts, detritus, sand grains, fish, macrophytes, *chlamydomonas species* and *spirogyra* (Ogari and Dadzie 1988; Njiru et al. 2004; Oso et al. 2006). *Rastrineobola argentea* feed on zooplankton and surface insects and migrate away from the shore after spending their larval stage in the shallow waters (Wanink 1999), with adults staying near the bottom during the day and rising to the surface at night. These references show how sampling for fish can be complex and given the size of Lake Victoria, their mobility and feeding habits can differentially affect their exposure to heavy metals and, thus, the results obtained in this study.

Cadmium showed significant ($p < 0.05$) seasonal and species variations with higher concentrations in all the three species during the wet season which would be due to higher concentrations in water and sediment as a consequence of wash-off (Ongeri et al. 2010). Cadmium mean concentrations followed the trend *Rastrineobola argentea* > *Oreochromis niloticus* > *Lates niloticus* during both wet and dry seasons. Lead (Pb) did not show significant ($p < 0.05$) seasonal variations but showed significant ($p < 0.05$) species variation with mean concentrations following the trend *Rastrineobola argentea* > *Lates niloticus* > *Oreochromis niloticus*. The Pb concentration levels recorded in the two *niloticus* species in both seasons were comparable with that recorded in *Tilapia nilotica* in a study of Lake Nasser in Egypt by Soltan et al. (2005) who found 0.48 µg/g dry weight. The Pb concentrations in all fish species were within the permissible limits of the FAO (1983) and the EC (2002) (Table 4). Copper showed significant species variations but no seasonal variation. The mean concentrations followed the trend *Lates niloticus* > *Rastrineobola argentea* > *Oreochromis niloticus* during both seasons. The mean Cu concentrations on dry weight basis for both seasons for the three fish species were much higher than the range of 0.2–0.5 µg/g Cu found in *Oreochromis niloticus* from Winam Gulf over 20 years ago (Ochieng 1987) and a mean concentration of 1.9 µg/g Cu recorded more recently by Kishe and Machiwa (2003) in *Oreochromis niloticus* sampled from Mwanza Gulf of Lake Victoria in Tanzania. The concentrations were much below the EC and FAO permissible limits for Cu. Zinc (Zn) and Fe showed no significant seasonal variations in *Lates niloticus and Oreochromis niloticus* samples but showed significant seasonal variations for *Rastrineobola argentea* samples. The concentrations of Zn in the two *niloticus* species were above the FAO permissible limits and its concentrations in *Rastrineobola argentea* were above both the EC and FAO permissible limits (Table 4). As expected, both seasons recorded Fe concentration levels in the three species of fish which were much higher than those recorded in *Oreochromis niloticus* from Winam Gulf in a previous study by Ochieng (1987), ranging from 1.0 to 6.4 µg/g dry weight which can be explained by the current status of pollution of the lake and more uptake and accumulation of the metal by fish lately. While the mean Fe concentration levels in both seasons were high in *Rastrineobola argentea* species, the results for the two *niloticus* species were relatively lower than the concentration levels of 69.9 and 89.8 µg/g for Fe recorded in *Tilapia galilea* and *Tilapia nilotica,* respectively, in a study of Lake Nasser by Soltan et al. (2005).

In general, the trend in the metal concentrations found was: Fe > Zn > Cu > Pb > Cd for *Lates niloticus* and *Oreochromis niloticus* and this seemed to agree with the trends reported in other studies (Arain et al. 2007). The

**Table 1** A summary of the mean (±SD) concentrations (μg/g dry weight) of heavy metals in three fish species from four sites at different seasons

| Site/species | Cd | | Pb | | Cu | | Zn | | Fe | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry |
| *Lates niloticus* | | | | | | | | | | |
| 1 | 0.18 ± 0.03 | 0.17 ± 0.03 | 0.85 ± 0.16 | 0.90 ± 0.28 | 2.95 ± 0.67 | 3.07 ± 0.27 | 42.4 ± 8.7 | 37.7 ± 4.4 | 73.3 ± 10.0 | 57.8 ± 2.3 |
| 2 | 0.22 ± 0.03 | 0.21 ± 0.03 | 0.71 ± 0.06 | 0.87 ± 0.06 | 4.55 ± 0.98 | 2.13 ± 0.21 | 38.2 ± 5.6 | 34.3 ± 5.2 | 37.4 ± 3.3 | 31.4 ± 4.5 |
| 3 | 0.28 ± 0.10 | 0.21 ± 0.12 | 1.16 ± 0.16 | 1.38 ± 0.10 | 4.18 ± 2.30 | 5.60 ± 2.04 | 39.7 ± 7.1 | 36.7 ± 4.2 | 50.8 ± 4.7 | 40.2 ± 8.0 |
| 4 | 0.21 ± 0.04 | 0.20 ± 0.04 | 0.47 ± 0.09 | 0.62 ± 0.04 | 2.56 ± 0.66 | 2.42 ± 0.22 | 33.2 ± 6.4 | 28.9 ± 4.5 | 44.2 ± 6.1 | 32.0 ± 1.2 |
| Site mean | 0.22 ± 0.04 | 0.20 ± 0.02 | 0.80 ± 0.39 | 0.94 ± 0.32 | 3.56 ± 0.95 | 3.30 ± 1.58 | 38.3 ± 3.8 | 34.4 ± 4.0 | 51.4 ± 15.6 | 40.0 ± 12.3 |
| *Oreochromis niloticus* | | | | | | | | | | |
| 1 | 0.21 ± 0.06 | 0.18 ± 0.03 | 0.76 ± 0.22 | 0.92 ± 0.06 | 2.17 ± 0.04 | 2.54 ± 0.57 | 34.2 ± 5.0 | 30.4 ± 2.5 | 51.9 ± 7.6 | 43.5 ± 2.9 |
| 2 | 0.33 ± 0.17 | 0.16 ± 0.05 | bdl | bdl | 2.31 ± 1.29 | 2.75 ± 0.40 | 42.5 ± 4.7 | 32.7 ± 4.0 | 53.3 ± 2.4 | 39.2 ± 2.2 |
| 3 | 0.25 ± 0.10 | 0.18 ± 0.04 | 0.69 ± 0.16 | 0.84 ± 0.12 | 2.89 ± 0.06 | 2.70 ± 0.12 | 35.3 ± 7.8 | 34.8 ± 3.4 | 59.5 ± 8.3 | 48.7 ± 2.7 |
| 4 | 0.21 ± 0.03 | 0.20 ± 0.02 | 0.76 ± 0.15 | 0.89 ± 0.04 | 3.56 ± 0.07 | 2.67 ± 0.25 | 47.0 ± 5.5 | 30.0 ± 5.4 | 47.6 ± 4.3 | 39.2 ± 2.1 |
| Site mean | 0.25 ± 0.06 | 0.18 ± 0.02 | 0.55 ± 0.37 | 0.66 ± 0.44 | 2.73 ± 0.63 | 2.67 ± 0.09 | 39.4 ± 6.1 | 32.5 ± 2.2 | 53.1 ± 4.9 | 42.9 ± 4.5 |
| *Rastrineobola argentia* | | | | | | | | | | |
| 1 | 0.26 ± 0.02 | 0.26 ± 0.13 | 2.36 ± 1.44 | 2.53 ± 0.09 | 8.74 ± 1.40 | 7.29 + 1.00 | 238.7 ± 68.0 | 214.2 ± 23.2 | 116.2 ± 20 | 70.0 ± 9.6 |
| 2 | 0.24 ± 0.03 | 0.22 ± 0.15 | 0.74 ± 0.05 | 0.74 ± 0.24 | 5.45 ± 2.31 | 5.97 + 0.95 | 409.3 ± 62.6 | 225.3 ± 7.4 | 137.1 ± 14 | 103.0 ± 67 |
| 3 | 0.40 ± 0.22 | 0.20 ± 0.01 | 0.69 ± 0.01 | 0.79 ± 0.03 | 7.17 ± 1.76 | 6.56 + 0.26 | 250.6 ± 64.8 | 207.1 ± 16.7 | 208.1 ± 4 | 207.3 ± 8 |
| 4 | 0.27 ± 0.12 | 0.24 ± 0.05 | 0.82 ± 0.65 | 0.64 ± 0.42 | 6.41 ± 2.20 | 4.52 + 0.72 | 239.4 ± 63.7 | 215.9 ± 38.7 | 156.6 ± 33 | 115.0 ± 2 |
| Site mean | 0.29 ± 0.07 | 0.23 ± 0.02 | 1.15 ± 0.80 | 1.18 ± 0.91 | 6.94 ± 1.39 | 6.08 + 1.18 | 284.5 ± 83.4 | 215.6 ± 83.4 | 154.5 ± 39 | 123.8 ± 60 |

*bdl* below detection limit

*Sites* 1: Sio Port, 2: Port Victoria, 3: Dunga, 4: Hippo Point

Wet: wet season; dry: dry season

**Table 2** Seasonal variations in mean concentrations (mean ± standard error) of heavy metals (µg/g dry weight) in fish from the four sampling sites combined

| Fish species | Wet season | Dry season | All season-all site means |
|---|---|---|---|
| *Lates niloticus* | | | |
| Cd | 0.22 ± 0.04 | 0.20 ± 0.02 | 0.21 ± 0.030 |
| Pb | 0.70 ± 0.29 | 0.94 ± 0.32 | 0.87 ±+0.30 |
| Cu | 3.56 ± 0.95 | 3.30 ± 1.58 | 3.43 ± 1.03 |
| Zn | 38.30 ± 3.80 | 34.4 ± 4.00 | 36.40 ± 3.90 |
| Fe | 51.40 ± 15.60 | 40.00 ± 12.30 | 45.70 ± 13.90 |
| *Oreochromis niloticus* | | | |
| Cd | 0.25 ± 0.06 | 0.18 ± 0.02 | 0.21 ± 0.02 |
| Pb | 0.55 ± 0.37 | 0.66 ± 0.44 | 0.61 ± 0.41 |
| Cu | 2.73 ± 0.63 | 2.67 ± 0.09 | 2.70 ± 0.33 |
| Zn | 39.40 ± 6.10 | 32.50 ± 2.20 | 35.90 ± 2.60 |
| Fe | 53.10 ± 4.90 | 42.90 ± 4.50 | 48.00 ± 4.50 |
| *Rastrineobola argentia* | | | |
| Cd | 0.29 ± 0.07 | 0.23 ± 0.02 | 0.26 ± 0.03 |
| Pb | 1.15 ± 0.80 | 1.18 ± 0.91 | 1.23 ± 0.85 |
| Cu | 6.94 ± 1.39 | 6.08 ± 1.18 | 6.51 ± 1.17 |
| Zn | 284.5 ± 83.40 | 215.60 ± 7.50 | 250.10 ± 44.90 |
| Fe | 154.5 ± 39.40 | 123.80 ± 58.80 | 139.10 ± 49.00 |

*Four sites* Sio Port, Port Victoria, Dunga and Hippo Point

*Number of seasons* 2 wet and 2 dry seasons (between May 2006 and Feb 2007)

*Total number of samples* 192

**Table 3** Seasonal variations in total mean concentrations of heavy metals (µg/g dry weight) in all the three finfish species samples combined

| All species (LN, ON, RA) | Wet season | Dry season | Combined mean |
|---|---|---|---|
| Cd | 0.26 ± 0.04 | 0.20 ± 0.03 | 0.23 ± 0.03 |
| Pb | 0.83 ± 0.30 | 0.93 ± 0.26 | 0.91 ± 0.31 |
| Cu | 4.41 ± 2.23 | 4.02 ± 1.82 | 4.21 ± 2.02 |
| Zn | 120.70 ± 141.80 | 94.20 ± 105.20 | 107.50 ± 123.50 |
| Fe | 86.30 ± 59.00 | 68.90 ± 47.60 | 77.60 ± 53.30 |

*LN, Lates niloticus, ON Oreochromis niloticus, RA Rastrineobola argentea*

Fe > Zn > Cu > Pb trend was also observed by Mzimela et al. (2003) in whole fish sampled in Mhlathuze Estuary in South Africa. In support of this trend, of all these five heavy metals analyzed, Fe has been found to be of highest concentration in both sediment and water in Winam Gulf, and Cd has been found to show the lowest concentration in both compartments (Ongeri et al. 2010). The trend observed in this study for *Rastrineobola argentea* was: Zn > Fe > Cu > Pb > Cd in both seasons which is the same as that found by Soltan et al. (2005) in a study in which whole fish specimens were analyzed. The finding of Soltan et al. (2005) also supports the higher concentrations of heavy metals in *Rastrineobola argentea* compared with other fish species as found in this study. Gills have been reported to have the highest concentrations of Cd, Cu and Zn, followed by scales, and then, muscles with lowest concentrations (Kishe and Machiwa 2003). In Table 3, the wet- and dry-season mean heavy metal concentrations recorded on dry weight basis, for the three fish species, were only slightly different from those found by Kishe and Machiwa (2003) in *Oreochromis niloticus* from Mwanza Gulf, Lake Victoria and Soltan et al. (2005) in *Tilapia nilotica* from Lake Nasser in Egypt. The wet- and dry-season means of heavy metal concentrations recorded on wet weight basis are compared with other levels obtained in previous studies as well as with the EU and FAO permissible limits in Table 4. The concentrations of Cd, Pb and Cu in all the fish species analyzed in this study appeared to be high but they were all lower than the EC and FAO permissible limits whereas Zn concentrations in all the three fish species exceeded the FAO permissible limits.

Pearson correlation analysis (data not shown) done on the trace metal concentrations on the three species of fish indicated significant positive correlation between Cu and Pb in *Lates niloticus* ($r = 0.91$, $p < 0.05$) and between Cu and Pb in *Rastrineobola argentea* ($r = 0.92$, $p < 0.05$) but the correlation between Cu and Pb in *Oreochromis niloticus* had a low negative value. The differences in correlations were perhaps, due to differences in feeding habits and physiological factors.

Overall, it is indicative from this study that *Rastrineobola argentea* can be a significant source of heavy metals especially Zn, to humans, compared with *Lates niloticus* and *Oreochromis niloticus* which are consumed after removal of their heads, scales and internal organs. In particular, more studies are needed to find out which specific organs of all three finfish species accumulate most heavy metal residues. The wet season contributed significantly to higher Cd, Cu, Zn and Fe concentrations in fish especially *Rastrineobola argentea* which could be due to higher concentrations of these metals in the lakewater during the rainy season, as a result of wash-off. However, Pb showed the reverse trend possibly due to the lower solubility of its salts in water.

**Table 4** Highest mean concentrations (μg/g wet weight) of heavy metals in fish obtained in this study compared with those obtained for other lakes and the EC and FAO permissible levels

| Site sampled | Species | Cd | Pb | Cu | Zn | Fe |
|---|---|---|---|---|---|---|
| Winam Gulf[a] | *Lates niloticus* | 48 | 205 | 777 | 8,360 | 11,223 |
| | *Oreochromis niloticus* | 53 | 140 | 581 | 8,383 | 11,298 |
| | *Rastrineobola argentea* | 57 | 228 | 1,374 | 56,336 | 30,594 |
| Winam Gulf[b] | Finfish (ranges) | 40–380 | 1,220–6,480 | 360–2,040 | 4,670–40,800 | 530–4,650 |
| Niger Delta[c] | Finfish (means) | 30 | 480 | 700 | 4,800 | 5,400 |
| [d]EC | ns | 100 | 400 | 10,000 | 50,000 | ng |
| [e]FAO | ns | ng | 500 | 30,000 | 4,500 | ng |

*nd* not determined, *ns* not specified, *ng* not given

[a] Present study 2006/7

[b] Wandiga and Onyari (1987)

[c] Kakulu et al. (1987)

[d] European Community (2002) permissible limits

[e] Food and Agriculture Organization (1983) permissible limits

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