Accumulation of Heavy Metals and their Effects on Antibiotic Resistance of Bacteria in an Aquaponics System

Hany M. Almotairy, Kevin Fitzsimmons and Jean E. McLain

Water evaporation and transpiration by plants can lead to concentration of salts and heavy metals in aquaponics systems. Developing countries tend to have higher concentrations of heavy metals in water supplies than developed countries and thus, aquaponics systems are potentially more at risk of heavy metal contamination in developing countries. Heavy metal contamination of fish and vegetables could present risks to human health.

Aquaponics is a promising, environmentally friendly and sustainable technology that contributes to food security and has the potential to alleviate poverty in many developing countries. Aquaponics technology consists of a recirculating water system where raising aquatic food animals (aquaculture) is combined with soilless growth of plant food crops (hydroponics). Fish and plants interact in a symbiotic manner, where fish provide nutrients for plants and in turn plants filter water to improve water quality conditions for fish.

Water evaporation and transpiration by plants can lead to concentration of salts and heavy metals in the system. This would be problematic if metals and metalloids become concentrated to the point where they exceed Maximum Contaminant Levels (MCLs) set by the USEPA for potable water quality. Developing countries tend to have higher concentrations of heavy metals in water supplies than developed countries (Jarup 2003) and thus, aquaponics systems are potentially more at risk of heavy metal contamination in developing countries. Heavy metal contamination of fish and vegetables could present risks to human health.

Heavy Metals

Heavy metals such as Cd, Pb, Hg, and As can threaten the sustainability of aquaponic systems (FAO 2014) because heavy metals may become toxic for living organisms, including bacteria that aid in nutrient cycling in aquaponic systems (Has-Schon *et al*. 2006, Schenone *et al*. 2014, Saha *et al*. 2015). Heavy metals are considered pollutants due to their toxicity, persistence in the environment and their ability to integrate and in some cases bioaccumulate within food chains (Klavins *et al*. 2000, Armitage *et al*. 2007, Sakan *et al*. 2009). Heavy metals can accumulate in fresh and marine water systems (Mendiguchia *et al*. 2006, Salami *et al*. 2008), posing a potential health concern for consumers and possibly leading to financial losses if concentrations in water exceed MCLs. Moreover, uptake of heavy metal contaminants may lead to concentrations in fish or edible plant tissue that exceed standards, contributing to economic losses, environmental degradation and negative human health impacts.

Accumulation of Heavy Metals

Heavy metals are used in many common agriculture and aquaculture practices as necessary micronutrients; metals are often added to animal feed, inorganic or organic fertilizers and pesticides, leading to increased heavy metal concentrations in the environment (Han *et al*. 2001, Burridge *et al*. 2010). Although heavy metals exist naturally in water bodies and bottom sediments (Engin *et al*. 2015), accumulation of heavy metals in water poses a risk to human and environmental health (Ali *et al*. 2013). Heavy metals tend to accumulate in sediment and thus heavy metal concentrations are often much higher in sediments than in water (Dummee *et al*. 2012). Aquatic organisms that live in polluted sediments often bioconcentrate heavy metals in their tissues (Li *et al*. 2009).

Fish can absorb water and compounds from sediments contaminated with heavy metals through the skin and gills (Ranasinghe *et al*. 2016). In aquaculture environments, Malik *et al*. (2010) evaluated the bioaccumulation of heavy metals, including Zn, Pb, Cd, Ni, Cu, Cr and Hg in freshwater fish tissues and found that different fish organs accumulated varying quantities of metals. Despite measured accumulation, the concentration of heavy metals was within maximum permissible standard values for human consumption. Laboratory experiments by Feldlite *et al*. (2008) showed no detectable levels of As, Cd, Hg, and Pb in fish flesh reared in recycled wastewater over two years, but levels of other heavy metals (Cd and Pb) in liver and bones of some fish were above the food standard.

In addition to fish, plants raised in aquaponics systems might also become contaminated with heavy metals. Heavy metals can accumulate in plant tissue, posing a potential health risk to consumers when ingested (Rana *et al*. 2011). As water from system circulates through plants (Miller *et al*. 1982), water loss occurs from the leaves through transpiration, but heavy metals do not transpire, resulting in accumulation of minute levels of metals in the leaf tissue over time. Contamination with heavy metals levels can damage plant cellular processes (Burzynski and Klobus 2004) and reduce plant size (Santala and Ryser 2009).

About 400 plant species are known as metal hyper-accumulators because they can accumulate metals in above-ground tissues (Kramer *et al*. 1997). Metal bioaccumulation by plants depends on pH, temperature and dissolved ions in water (Engin *et al*. 2015). Crews and Davies (1985) grew six different lettuce species in soils contaminated with various concentrations of metals Cd, Cu, Pb, and Zn and the uptake of Cd and Zn increased with increasing concentration in the soil and there was a significant correlation for accumulation of both metals in lettuce leaves.

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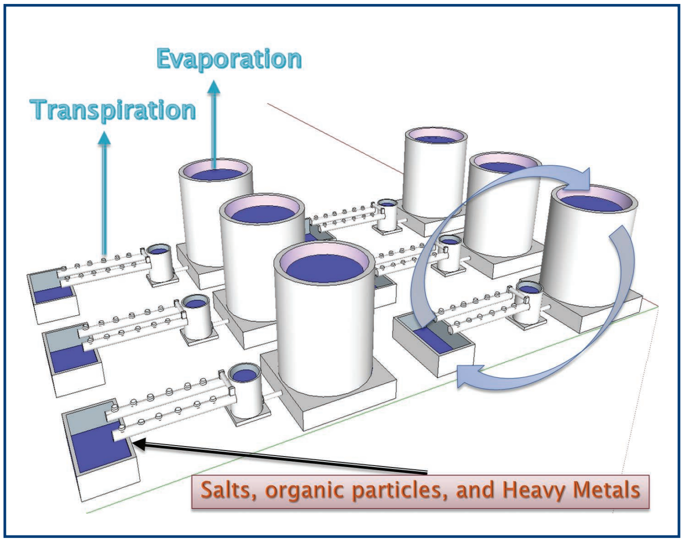

FIGURE 1. *Aquaponics system design. Three systems were dosed with heavy metals (treatment) and three were not (control). Water can be lost by evapotranspiration, but heavy metals might be concentrated with time.*

FIGURE 2. *The main components of the systems for each replicate. From left to right: sump, lettuce growing hydroponically using nutrient film technique, biofilter, and fish tank. Water is pumped from the sump to the fish tank and then flows by gravity to the biofilter, lettuce tray and sump.*

Bacterial Antibiotic Resistance

Bacterial antibiotic resistance is common in the environment, and is especially prevalent in water that is affected by pollutants resulting from human activities (Di Cesare *et al*. 2016). For instance, using antimicrobials for controlling disease in fish has long been a common activity in some countries; this practice is often used during international transportation of fish to inhibit growth of potential pathogens (Verner-Jeffreys *et al*. 2009).

In recent decades, reports from The World Health Organization (WHO), National Institutes of Health (NIH), Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC) state that drug-resistant bacteria are posing a serious threat to human health. The rapid spread of antibioitc-resistant bacteria has resulted in widespread difficulty in treating common infections in humans and animals (Levy and Marshall 2004, Bax and Griffin 2012, Di Cesare *et al*. 2016). Contaminated water and food supplies appear to be helping to move antibiotic resistance into different environments (Suzuki and Hoa 2012). The factors that are driving selection for bacterial resistance are still largely unclear (Di Cesare *et al*. 2016) but heavy metals have the ability to play a potential role in enhancing co-selection for antibiotic resistance. In short, heavy metal pollutants increase bacterial tolerance to antibiotics related to co-regulation of resistance genes (Baker-Austin *et al*. 2006).

The Experiment

A 12-wk experiment was conducted to assess the partitioning and potential bioaccumulation of heavy metals in water, fish and plant tissue of an aquaponics system. The growth of aquaponics worldwide and the widespread presence of heavy metals in water sources indicates that there is a need to identify any potential health risks to consumers. Co-selection of antibiotic resistance in bacteria within aquaponics systems may also present a potential hazard to human health and the environment.

To examine the distribution of heavy metals and the potential for antibiotic resistance development, an experiment was carried out in a greenhouse at the Controlled Environment Agriculture Center of the University of Arizona. A small-scale aquaponics system was developed with Nutrient Film Technique as the hydroponic method (Figs. 1 and 2). Systems were stocked with eight Nile tilapia *Oreochromis niloticus* fingerlings and ten Butterhead variety lettuce *Lactuca sativa*. Fish were fed at 2.5 percent body weight daily. Systems were operated for six weeks before initating the experiment.

Three systems did not receive metal supplementation (control). As the background levels of heavy metals in potable water and fish feed are low, metals were added to three systems (treatment). Based on the MCL for potable water standards of the US Environment Protection Agency (EPA 2016), metals were added to reach a target of 60 percent of the MCL of four heavy metals (Cd, Pb, Hg and As). Heavy metal concentrations were determined in water samples collected every week. Samples of fish and plants were collected on the first and final days of the experiment. In addition, weekly water samples were collected for culturing bacteria to evaluate antibiotic resistance. Inductively coupled plasma mass spectrometry (ICP-MS) was performed to determine concentrations of heavy metals in samples of water, fish, and plants. Agar plating was used to evaluate bacterial antibiotic resistance.

Heavy Metals in Water

Concentrations of the four metals in the aquaponic system water varied over time. In general, concentrations of As were greater than those of other metals (Cd, Hg and Pb) due to high background levels and increased with time. Also, the As concentration of fish feed (3.03 μg/g feed) was approximately an order of magnitude greater than that of Cd (0.37 μg/g feed), Hg (0.80 μg/g feed) and Pb (0.30 μg/g feed), resulting in an increase in water As concentration over time (Fig. 3). Mercury levels decreased over time in both the treated and the control systems (Fig. 4). Cadmium and lead concentrations fluctuated over time, but concentrations were maintained within the MCL (5 μg/L). Lead concentrations were low in water of both treatment and control systems. At the end of the experiment, Pb concentrations were less than immediately after heavy metal addition to the system.

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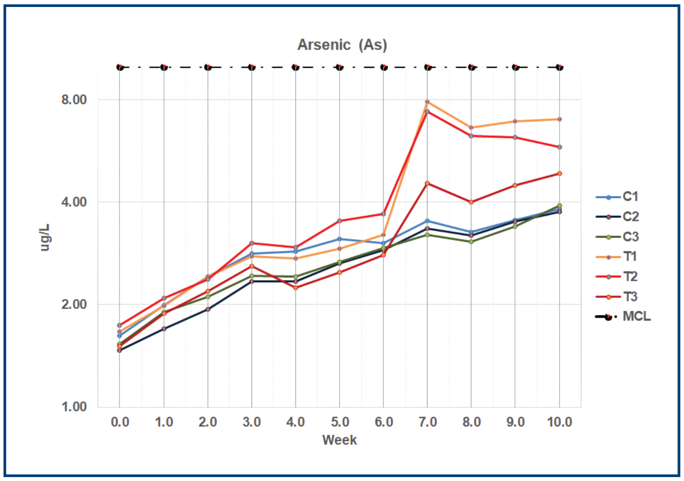


FIGURE 3. *Arsenic concentration in aquaponics system water over time.*

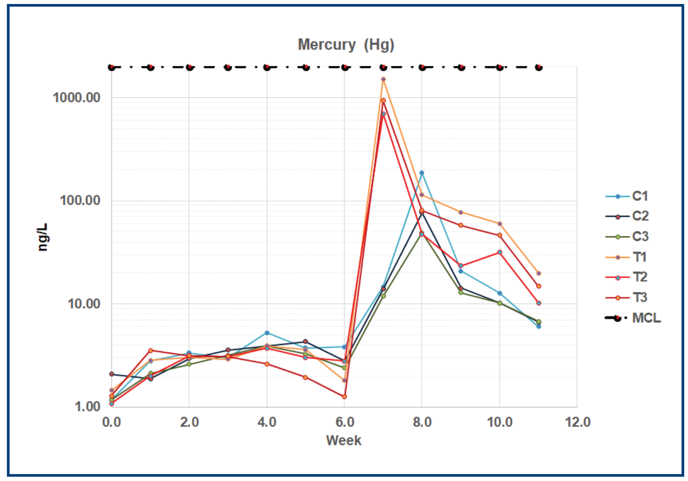


FIGURE 4. *Mercury concentration in aquaponics system water over time.*

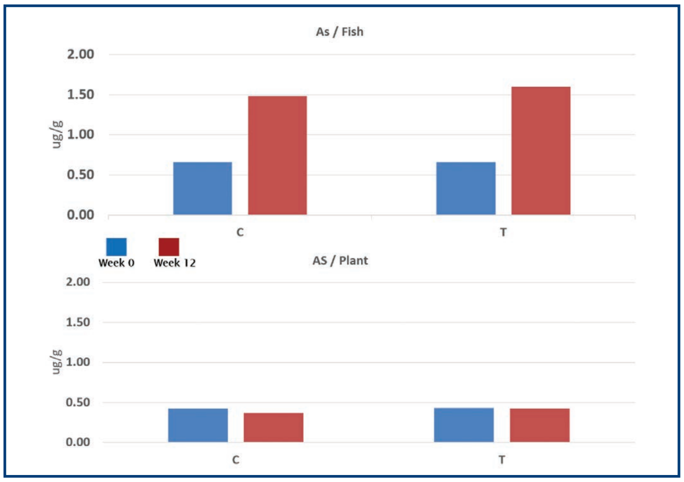


FIGURE 5. *Change in arsenic concentration in fish and plant tissue between the beginning and the end of the study.*

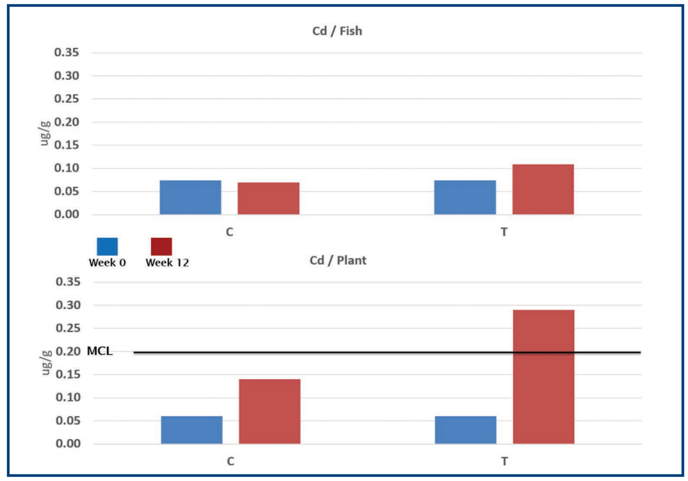


FIGURE 6. *Change in cadmium concentration in fish and plant tissue between the beginning and the end of the study.*

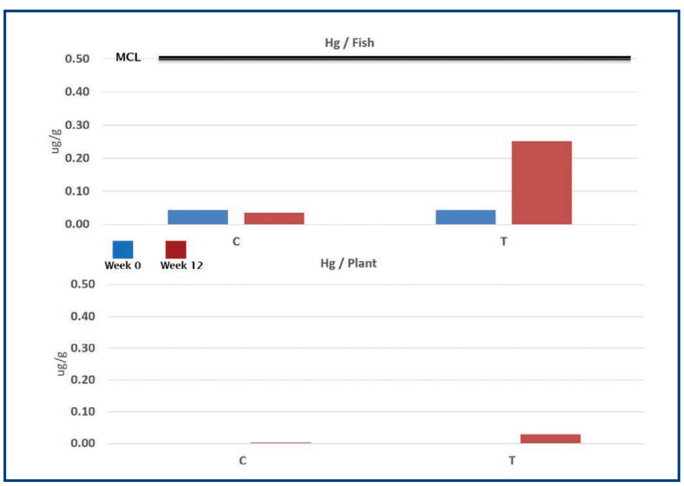


FIGURE 7. *Change in mercury concentration in fish and plant tissue between the beginning and the end of the study.*

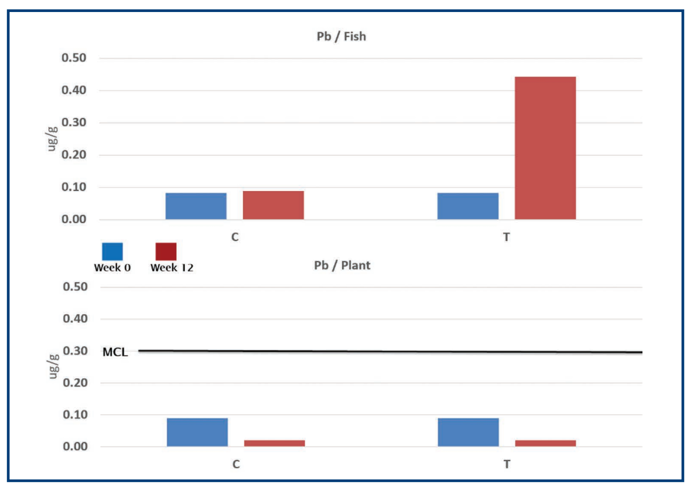


FIGURE 8. *Change in lead concentration in fish and plant tissue between the beginning and the end of the study.*

CONCENTRATIONS OF AS WERE GREATER THAN THOSE OF OTHER METALS (CD, HG AND PB) DUE TO HIGH BACKGROUND LEVELS AND INCREASED WITH TIME. MERCURY LEVELS DECREASED OVER TIME IN BOTH THE TREATED AND THE CONTROL SYSTEMS. CADMIUM DID NOT ACCUMULATE IN FISH TISSUES IN TREATED OR CONTROL SYSTEMS BUT ACCUMULATED IN LETTUCE IN BOTH CONTROL AND TREATMENT SYSTEMS.

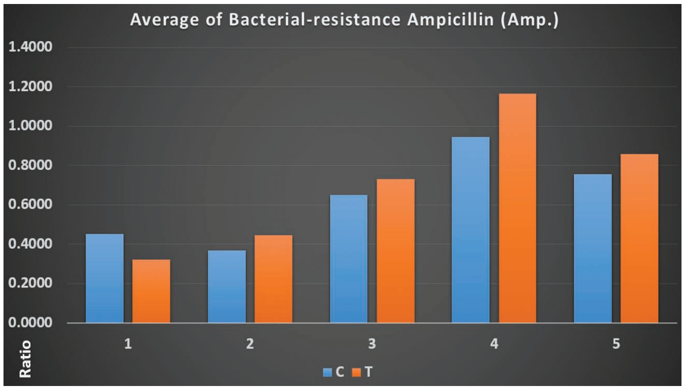
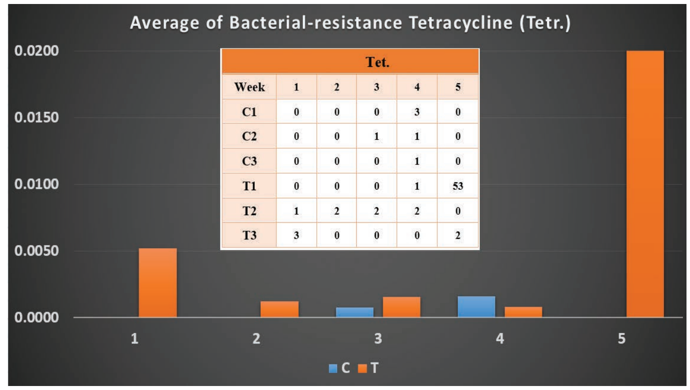


FIGURE 9. *Bacterial-resistance to ampicillin (Amp.). X axis represents the last five weeks of the experiment, after heavy metals were added to systems. Y axis represents the ratio of bacterial counts of the average of Control system replicates (blue) and the average of the Treatment system replicates (orange) to a control with no antibiotic.*



Tet.					
Week	1	2	3	4	5
C1	0	0	0	3	0
C2	0	0	1	1	0
C3	0	0	0	1	0
T1	0	0	0	1	53
T2	1	2	2	2	0
T3	3	0	0	0	2

FIGURE 10. *Bacterial-resistance to tetracycline (Tetr.). X axis represents the last five weeks of the experiment, after heavy metals were added to systems. Y axis represents the ratio of bacterial counts of the average of Control system replicates (blue) and the average of the Treatment system replicates (orange) to a control with no antibiotic.*

Heavy Metals in Fish and Plant Tissues

Samples of fish and plant tissues were tested for heavy metal concentrations on the first and last days of the experiment. The standards for heavy metals in fish and plant tissues, based on Codex Alimentarius food standards, were available only for Cd and Pb in leafy vegetables and Hg in fish. The difference in heavy metal concentrations between the first and last day indicated that fish accumulated As in their tissues, but lettuce did not (Fig. 5). Cadmium did not accumulate in fish tissues in treated or control systems but accumulated in lettuce in both control and treatment systems, and lettuce in treatment systems was contaminated with Cd above the MCL (Fig. 6). Mercury accumulated only in fish tissues in the metal-treated system, but concentrations remained within the safe range (Fig. 7). Lead did not accumulate in fish in the control system but accumulated to greater levels in the treatment systems. However, Pb did not accumulate in plant tissues in treatment or control systems (Fig. 8).

Antibiotic Resistance

Bacteria counts were conducted from the water samples of the treated and control systems to identify any correlations between heavy metals and bacterial resistance to ampicillin and tetracycline. Samples were collected weekly over the last five weeks of the experiment. Bacterial resistance to both antibiotics showed a general increase over time in systems treated with heavy metals (Figs. 9 and 10), but these increases were not significant compared to control. In general, there was greater resistance to ampicillin than to tetracycline.

Conclusions and Future Work

The low levels of metals added to the treatment systems did not reduce the quality of the fish or plants. No exceedances of MCLs were revealed in the food products for the metals tested (As, Hg and Pb), except for Cd in lettuce. The microbial results indicated a slight trend of resistance development to ampicillin and tetracycline over time, but these results were not statistically signficant.

These initial experiments will guide future work that may shed more light on heavy metal accumulations in aquaponics and the development of antibiotic resistance. Future work will include extending experiment time with several cycles of plant and fish growth, spiking with higher levels of heavy metals that are equal to concentrations found in water sources in developing countries, testing bacterial resistance to different antibiotics and preparing a mass balance model to identify the partitioning and exact accumulation of heavy metals within the aquaponics system.

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Notes

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References

Ali, H., K. Ezzat and A.S. Muhammad. 2013. Phytoremediation of heavy metals concepts and applications. Chemosphere 91:869-881.

Armitage, P.D., M.J. Bowes and H.M. Vincent. 2007. Long-term changes in macroinvertebrate communities of a heavy metal polluted stream: The River Nent (Cumbria. UK) after 28 years. River Research 23:997-1015.

Baker-Austin, C., M.S. Wright, R. Stepanauskas and J.V. McArthur. 2006. Co-selection of antibiotic and metal resistance. Trends Microbiology 14:176-182.

Bax, R. and D. Griffin. 2012. Introduction to antibiotic resistance. Pages 1-12 *In*: A.R.M. Coates. Antibiotic Resistance. Springer Heidelberg, New York, NY USA.

Burridge, L., J.S. Weis, F. Cabello, J. Pizarro and K. Bostick. 2010. Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306:7-23.

Burzynski, M. and G. Klobus. 2004. Changes of photosynthetic

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parameters in cucumber leaves under Cu, Cd, and Pb stress. Photosynthetica 42:505-510.

Crews, H.M. and B.E. Davies. 1985. Heavy metal uptake from contaminated soils by six varieties of lettuce (*Lactuca sativa L.*). The Journal of Agricultural Science 105:591-595.

Di Cesare, A., E.M. Eckert and G. Corno. 2016. Co-selection of antibiotic and heavy metal resistance in freshwater bacteria. Journal of Limnology 75(2S):59-66.

Dummee, V., M. Kruatrachue, W. Trinachartvanit, P. Tanhan, P. Pokethitiyookand P. Damrongphol. 2012. Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail in Beung Boraphet Reservoir, Thailand. Ecotoxicology and Environmental Safety 86:204-212.

Engin, M.S., A. Uyanik and H.G. Kutbay. 2015. Accumulation of heavy metals in water, sediments and wetland plants of Kizilirmak Delta (Samsun, Turkey). International Journal of Phytoremediation 17:66-75.

EPA (U. S. Environmental Protection Agency). 2016. Ground water and drinking water. Table of Regulated Drinking Water Contaminants. www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants#one .

Feldlite, M., M. Juanico, I. Karplus and A. Milstein. 2008. Towards a safe standard for heavy metals in reclaimed water used for fish aquaculture. Aquaculture 284:115-126.

FAO (Food and Agriculture Organization of the United Nations). 2014. Small-scale aquaponic food production: Integrated fish and plant farming, 00153 Rome, Italy.

Han, F., W. Kingery and H. Selim. 2001. Accumulation, redistribution, transport and bioavailability of heavy metals in waste amended soils. Pages 141-168 *In:* I. Iskander and M. Kirkham, editors. Trace Elements in Soil: Bioavailability, Fluxes and Transfer. CRC, Boca Raton, FL USA.

Has-Schon, E., I. Bogut and I. Strelec. 2006. Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva (Croatia). Archives of Environmental Contamination and Toxicology 50:545-551.

Jarup, L. 2003. Hazards of heavy metal contamination. British Medical Bulletin 68(1):167-182.

Klavins M., A. Briede, V. Rodinov, I. Kokorite, E. Parele and I. Klavina. 2000. Heavy metals in rivers of Latvia. Science of the Total Environment 262:175-183.

Kramer, U., R.D. Smith, W.W. Wenzel, I. Raskin and D.E. Salt. 1997. The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense* Halacsy. Plant Physiology 115:1641-1650.

Levy, S.B. and B. Marshall. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine 10:S122-S129.

Li, X., L. Jia, Y. Zhao, Q. Wang and Y. Cheng. 2009. Seasonal bioconcentration of heavy metals in *Onchidium struma* (Gastropoda: Pulmonata) from Chongming Island, the Yangtze Estuary, China. Journal of Environmental Science 21:255-262.

Malik, N., A.K., Biswas, T.A. Qureshi, K. Borana and R. Virha. 2010. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. Environmental Monitoring and Assessment 160:267-276.

Mendiguchia, C., C., Moreno, M.P. Manuel-Vez and M. Garcia-Vargas. 2006. Preliminary investigation on the enrichment of heavy metals in marine sediments originated from intensive aquaculture effluents. Aquaculture 254:317-325.

Miller, R.F., F.A. Branson, I.S. McQueen and C.T. Snyder. 1982. Water relations in soils as related to plant communities in Ruby Valley, Nevada. Journal of Range Management 35:462-468.

Ranasinghe, P., S. Weerasinghe and M.N. Kaumal. 2016. Determination of heavy metals in tilapia using various digestion methods. International Journal of Scientific Research and Innovative Technology, Sri Lanka. 3(6):38-48.

Rana, S., S.K. Bag, D. Golder, S. Mukherjee (Roy), C. Pradhan and B.B. Jana. 2011. Reclamation of municipal domestic wastewater by aquaponics of tomato plants. Ecological Engineering 37:981-988.

Saha, S., G.C. Hazra, B. Saha and B. Mandal. 2015. Assessment of heavy metals contamination in different crops grown in long-term sewage-irrigated areas of Kolkata, West Bengal, India. Environmental Monitoring and Assessment 187:1-12.

Sakan, S.M., D.S. Dordevic, D.D. Manojlovic and P.S. Predrag. 2009. Assessment of heavy metal pollutants accumulation in the Tisza River sediments. Journal of Environmental Management 90:3382-3390.

Salami, I.R.S., S. Rahmawati, R.I.H. Sutarto and P.M. Jaya. 2008. Accumulation of heavy metals in freshwater fish in cage aquaculture at Cirata Reservoir, West Java, Indonesia. Annals of the New York Academy of Sciences 1140:290-296.

Santala, K.R. and P. Ryser. 2009. Influence of heavy-metal contamination on plant response to water availability in white birch, *Betula papyrifera*. Environmental and Experimental Botany 66:334-340.

Schenone, N.F., L. Vackova and A. Fernandez Cirelli. 2014. Differential tissue accumulation of arsenic and heavy metals from diets in three edible fish species. Aquaculture Nutrition 20:364-371.

Suzuki, S. and P.T.P. Hoa. 2012. Distribution of quinolones, sulfonamides, tetracyclines in aquatic environment and antibiotic resistance in Indochina. Frontiers in Microbiology 3, 67. 10.3389/fmicb.2012.00067.

Verner-Jeffreys, D.W., T.J. Welch, T. Schwarz, M.J. Pond, M.J. Woodward, S.J. Haig, and C. Baker-Austin. 2009. High prevalence of multidrug-tolerant bacteria and associated antimicrobial resistance genes isolated from ornamental fish and their carriage water. Plos One 4(12):e8388.