AMARANTHUS HYBRIDUS IN PHYTOREMEDIATION OF HEAVY METAL POLLUTED WATERS OF NAIROBI RIVER

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Abstract
Nairobi River has high quantities of heavy metals emanating mainly from industrial and domestic wastes. Phytoremediation is a promising alternative to conventional clean-up methods; however, not enough information is available on plant species suitable for this application. Plant screening on contaminated sites can lead to the identification of more species. A phytoremediation study was carried out along Nairobi River in six sites; Kikuyu, Kawangware, Chiromo, Gikomba, Njiru, and Fourteen falls. The objective of this study was to ascertain the extent of heavy metal pollution and the potential of Amaranthus hybridus (A. hybridus) as a phytoremediant. The heavy metals studied were Copper (Cu), Cadmium (Cd) and Zinc (Zn). The heavy metals present in A. hybridus (biotic indicator), water and soil (abiotic indicators) were detected using the atomic absorption spectrophotometer (AAS). The observed values of heavy metals in water, soil and A. hybridus did not vary significantly (p>0.05) among the sampling sites. The concentration of these metals in soil (42.88 ± 0.03 mg/Kg) was higher than the values recorded in water (37.61 ± 0.65 mg/L). Based on the concentration observed in A. hybridus; Cd - 4.19 ±0.15 mg/Kg, Cu –8.73 ±0.5 mg/Kg and Zn - 17.42 ± 2.4 mg/Kg, Zn > Cu > Cd. Zn showed the highest accumulation and can be considered as one of the major pollutants in Nairobi River. Bio-concentration factor obtained was 8.44 ± 0.06. This study showed that A. hybridus can accumulate heavy metals even when the concentration of the metals in the abiotic components is low; suggesting that it can be used in phytoremediation of heavy metal polluted aquatic ecosystems.

Key words: phytoremediation, Amaranthus hybridus, heavy metal bio-concentration

1.0 Introduction
1.1 Study Area
The Nairobi River and its tributaries traverse through the Nairobi County which is the Kenyan Capital. It is the main river of the Nairobi River Basin, a complex of several parallel streams flowing eastwards i.e. Ngong River, Mathare River and Motoine River. All of them join east of Nairobi and meet the Athi River, eventually flowing to the Indian Ocean. These rivers are mostly narrow and highly polluted. The main stream, Nairobi River, bounds the northern city centre and is partly canalized. Nairobi River lies between 1° 11’ 59” S and 37° 9’ 26” E (Figure 1).

Ecologically, the study area lies within agro-ecological zones which range from humid, through semi-humid to semiarid lands. However, land-use systems are highly influenced by rainfall patterns, topography and human activities. The area has two distinctive land-use systems, comprising agriculture, which is the main land use in the Kiambu area, and industry, which is the predominant land use in Nairobi city and its environs. Population growth and industrial production has increased the volume of domestic waste and effluent load discharged into the rivers passing through the city and has caused a serious deterioration in water quality. The source of Nairobi River is the Kikuyu springs at an altitude of 2000 m above sea level. From Kikuyu the river flows eastwards through Dagoretti, Kawangware, Chiromo, the central business district, Eastleigh and Kariobangi sewage treatment works. After Kariobangi the Nairobi River runs through barren Njiru quarry sites where the Gitathuru and Ruaraka Rivers join it. The Nairobi River then flows past the Nairobi Falls and Fourteen Falls. The river joins the Athi River and eventually the Sabaki River which discharges its water into the Indian Ocean at Malindi on the East African coast.
1.2 Sources of pollution in Nairobi River

Water pollution is mainly a result of human activities and makes the water dangerous to human beings, unfit for industrial use and adversely affects the aquatic biota. Water pollution is associated with human population explosion and industrialization. The main sources of water pollution are industrial discharge, sewage, agricultural waste, fertilizers, and seepage from waste sites, decaying plant life, road, railway and sea accidents involving large oil carriers (Kinchella and Hyland, 1993). The city has experienced rapid industrialization and growth in population during the last 100 years (Okoth and Otieno, 2001). This rapid growth has not been matched by development of infrastructure to deal with waste disposal. As a result problems have arisen with regard to garbage, human and industrial waste disposal leading to pollution of the water resources. Sources of pollution of the Nairobi River include industrial effluent, effluent from petrol stations and motor vehicle garages, surface run off, factories and other business premises, raw sewage from broken or overloaded sewers as well as raw sewage from informal settlements (Ndwaru, 1994; Otieno, 1995; Okoth and Otieno, 2001).

1.3 Environmental issues facing Nairobi River

Pollution of rivers and streams is one of the crucial environmental problems. Although some kinds of water pollution can occur through natural processes, it is mostly as a result of human activities. Kenya’s scarce water resources are under threat from pollution with the major sources of pollution being domestic/municipal and industrial. Human settlements along the Nairobi River have increased dramatically due to the growth of the city and population increase. This has raised serious concern for the environmentalists on the state of Nairobi River for several decades. The River has seen a massive deterioration in quality with the increase in population of the city. This increase in the city’s population coupled with a sluggish economy has led to the mushrooming of slums, which tend to reside next to the riverbanks of which sanitation facilities are non-existent. Since the communities are not served by amenities and public utilities, they have tended to discharge their raw sewage into the streams next to them. This has led to the well-being of communities living downstream of the Nairobi River being adversely affected. Also lack of environmental awareness and law enforcement capacity has left Nairobi residents with a deplorable situation impacting adversely on all who live in, or indeed visit the city.

1.4 AmaranthusHybridus

*Amaranthushybridus* (*A. hybridus*) (Figure 2), commonly called smooth amaranth, smooth pigweed, red amaranth, or slim amaranth, is a species of annual flowering plant. *A. hybridus* grows to 2m (6ft 7in). It is frost tender. It is in flower from July to September. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind, self. The plant is self-fertile. Suitable for: light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. Suitable pH: acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It prefers moist soil. No members of this genus are known to be poisonous, but when grown on nitrogen-rich soils they are known to concentrate nitrates in the leaves. This is especially noticeable on land where chemical fertilizers are used. Nitrates are implicated in stomach cancers, blue babies and some other health problems. It is inadvisable, therefore, to eat this plant if it is grown inorganically.
1.5 Phytoremediation

Phytoremediation, an emerging cleanup technology for contaminated soils, groundwater, and wastewater that is both low-tech and low-cost, is defined as the engineered use of green plants (including grasses, forbs, and woody species) to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water. This definition includes all plant-influenced biological, chemical, and physical processes that aid in the uptake, sequestration, degradation, and metabolism of contaminants, either by plants or by the free-living organisms that constitute the plant’s rhizosphere. Phytoremediation takes advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body. Several comprehensive reviews have been written on this subject, summarizing many important aspects of this novel plant-based technology. The basic idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source. However, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising, cost-effective, and environmentally friendly technology. Phytoremediation can be applied to both organic and inorganic pollutants, present in solid substrates (e.g. soil), liquid substrates (e.g. water), and the air (Lone et al., 2008).

1.6 Case Studies

Several aquatic species have been identified and tested for the phytoremediation of heavy metals from the polluted water. These include sharp dock (*Polygonum amphibium* L.), duck weed (*Lemma minor* L.), water hyacinth (*Eichhornia crassipes*), water lettuce (*P. stratiotes*), water dropwort (*Oenanthe javanica*), calamus (*Lepironia articulata*), pennywort (*Hydrocotyle umbellata* L.) (Prasad and Freitas, 2003). The roots of Indian mustard are found to be effective in the removal of Cd, Cr, Cu, Ni, Pb and Zn, and sunflower can remove Pb, U, Cs-137 and Sr-90 from hydroponic solutions (Zaranyika and Ndapwadza, 1995; Wang et al., 2002; Prasad and Freitas, 2003). The potential of duck weed was investigated by Zayed et al., 1998 for the removal of Cd, Cr, Cu, Ni, Pb and Se from nutrient-added solution and the results indicate that duck weed is a good accumulator for Cd, Se and Cu, a moderate accumulator for Cr, but a poor accumulator of Ni and Pb. Dos Santos and Lenzi, 2000 tested aquatic macrophyte (*Eichhornia crassipes*) in the elimination of Pb from industrial effluents in a greenhouse study and found it useful for Pb removal. Wang et al., 2002 conducted a pot experiment to test five wetland plant species, i.e., sharp dock, duckweed, water hyacinth, water dropwort and calamus for their possible use in remedying the polluted waters. The results show that sharp dock was a good accumulator of N and P. Water hyacinth and duckweed strongly accumulated Cd with a concentration of 462 and 1420 mg/Kg, respectively. Water dropwort achieved the highest concentration of Hg, whereas the calamus accumulated Pb (512 mg/Kg) substantially in its roots. Other studies show that microorganisms, genetically modified organisms, bacteria and algae can also be used to remediate polluted sites.
2.0 Materials and Methods
All reagents (hydrochloric, HCl, and nitric, HNO₃ acids) used were of analytical grade. All glassware were washed and rinsed with 10% HCl followed by distilled water to avoid metal contamination. Sample preparation and analysis were carried out using standard methods of analysis (Association of Official Analytical Chemists - AOAC).

2.1 Sampling and Sampling Sites
Soil and water samples were collected in triplicate from six sampling sites; Kikuyu (site 1), Kawangware (site 2), Chiromo (site 3), Gikomba (site 4), Njiru (site 5) and Fourteen Falls (site 6). A. hybridus was collected from Nairobi River along the river bank (Figure 3). Samples were collected during the months of February and March 2012, the dry season only. The sites were chosen considering relevance as point sources of pollution, assumed mid-point of the river, along feeder river discharge canals and the source to serve as a control.

2.2 Sample pre-treatment
Water samples were collected as grab samples in pre-cleaned containers in triplicates from all sites and treated with nitric acid (2%); these were stored in a cool box and transported to the laboratory. Soil samples, approximately 500g were scooped with a shovel at a maximum depth of 10 cm and homogenized before a laboratory sample was drawn. Samples were stored in polythene bags. The aquatic plant was collected by hand, washed with river water to remove sediment particles, placed in a plastic bag, labeled carefully and brought to the laboratory. Polythene tools were used in sampling and storing the collected matrices to avoid metal contamination.

Figure 3: Sampling sites

2.3 Water Samples
Water samples were thoroughly mixed and aliquots of 50 ml taken in triplicates. These were acid digested with nitric acid until clear solutions were obtained; digests were filtered with Whatman No. 42 and stored in plastic bottles.
2.4 Plant Samples
The plant sample was dried in an oven (WTB Binder) at 105°C then crushed using pestle and mortar for further analysis. Samples were wet digested, extracted with nitric acid and filtered through Whatman filter paper No. 42. The digests were then diluted to 100 ml with distilled water.

2.5 Soil Samples
Soil samples were air dried, then crushed in a mortar and pestle and sieved through 2 mm governorates sieve to remove plant parts and debris. Well mixed samples of approximately 2g each, weighed using a digital analytical balance (Mettler Toledo) with an accuracy of 0.001g, were placed in 250 ml glass beakers and wet digested with nitric acid until clear solutions were realized. The resulting solutions were filtered using Whatman filter paper no. 42 and then diluted to 100 ml with distilled water. The digests were stored in the refrigerator in pre-cleaned containers and analysis carried out within one week.

2.6 AAS Analysis
Flame atomic absorption spectrophotometer (AAS - Shimadzu AA 7000) was used in the analysis. The concentrations of the metals were determined in triplicates. The accuracy and precision of the analytical procedure were determined. A series of standards were prepared for instrumental calibration by serial dilution of working solutions (100 mg/l) prepared from analytical grade stock solutions (1000 mg/l) from Sigma and Aldrich INC., USA. For the elements (Cu, Zn, Cd) six standard solutions of different concentrations were prepared in 0.1M HNO₃ within linear concentration range (Table 2-1). The calibration curves were prepared for each of the metals investigated by least square fitting. Quality assurance was guaranteed through triple determinations and use of blanks for correction of background and other sources of error.

2.7 Data Analysis
The concentrations of heavy metals in various matrices were presented as arithmetic mean with standard deviation (mean ± standard deviation). Statistical analyses were done at p = 0.05 (Miller and Miller, 1998). The Bio - concentration factor (BCF) was also calculated (Zayed et al., 1998).

\[ BCF = \left( \frac{P}{E} \right)_i \]

Where \( i \) denote the heavy metal and BCF is the bio-concentration factor and is dimensionless. \( P \) represents the trace element concentration in plant tissues (mg/Kg dry weight); \( E \) represents the trace element concentration in the water (mg/l). The results are presented in tables.

Table 1: Concentration of calibrating standards

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration of calibrating standards, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0, 0.2, 0.4, 0.6, 0.8 and 1.0.</td>
</tr>
<tr>
<td>Zn</td>
<td>0, 0.2, 0.4, 0.6, 0.8 and 1.0.</td>
</tr>
<tr>
<td>Cd</td>
<td>0, 0.02, 0.04, 0.06, 0.08 and 0.1</td>
</tr>
</tbody>
</table>

3.0 Results and Discussion

3.1 Heavy metal concentration in water, soil and A. hybridus
The concentrations of Cu, Zn and Cd in water, soil and A. hybridus are presented in Table 1. The mean concentration of Cu in water, soil and A. hybridus in the six sites (Kikuyu, Kawangware, Chiromo, Gikomba, Njiru and Fourteen Falls) were similar and there was no significant difference (p>0.05).

The mean concentration of Zn in water ranged from 30.58 ± 5.5 mg/L in site 1 to 85.64 ± 8.2 mg/L in site 4 (Table 3-1). This difference is significant (p<0.05). The highest mean concentration (101.73 ± 10.5 mg/Kg) of Zn in soil was recorded in site 5 while the lowest value (35.25 ± 6.2 mg/Kg) was recorded in site 1. This difference was also significant (p<0.05). Site five had the highest mean concentration (21.88 ± 3.3 mg/Kg) of Zn recorded in A. hybridus while the lowest concentration (8 ± 0.5 mg/Kg) was recorded in site 1. Again this difference is significant (p<0.05). The high Zn content could be attributed to the sewage treatment plant or quarry activity in the area.

The range of concentration of Cd in the waters of Nairobi River varied from 21.58 ± 4.6 mg/L in site 1 to 40.35 ± 3.2 mg/L in site 4 (Table 2). The highest mean concentration (49.68 ± 6.3 mg/Kg) of Cd in soil was recorded in
site 5 while the lowest mean concentration (22.71 ± 4.5 mg/Kg) occurred in site 1. Site 3 recorded the highest mean concentration (8.28 ± 0.6 mg/Kg) of Cd in A. hybridus while the lowest value (1.02 ± 0.1 mg/Kg) was obtained in A. hybridus found in site 2. This differences in the concentrations of Cd in water, soil and A. hybridus were not significant (p>0.05) among the sampling sites.

Table 2: Concentration of Cu, Zn and Cd in soil, water and A. hybridus

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Water (mg/L)</th>
<th>Soil (mg/L)</th>
<th>A. hybridus (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site /Element</td>
<td>Cu</td>
<td>Zn</td>
<td>Cd</td>
</tr>
<tr>
<td>Site 1</td>
<td>17.15 ± 2.5</td>
<td>30.58 ± 5.5</td>
<td>21.58 ± 4.6</td>
</tr>
<tr>
<td>Site 2</td>
<td>18.35 ± 2.4</td>
<td>40.38 ± 7.5</td>
<td>25.78 ± 4.8</td>
</tr>
<tr>
<td>Site 3</td>
<td>23.25 ± 4.4</td>
<td>39.54 ± 7.1</td>
<td>37.85 ± 4.6</td>
</tr>
<tr>
<td>Site 4</td>
<td>28.65 ± 4.3</td>
<td>85.64 ± 8.2</td>
<td>40.35 ± 3.2</td>
</tr>
<tr>
<td>Site 5</td>
<td>25.65 ± 3.6</td>
<td>80.54 ± 8.1</td>
<td>36.28 ± 3.9</td>
</tr>
<tr>
<td>Site 6</td>
<td>25.65 ± 3.5</td>
<td>70.95 ± 7.6</td>
<td>28.69 ± 2.6</td>
</tr>
</tbody>
</table>

The three investigated heavy metals (Cu, Zn and Cd) were detected in measurable quantities in Nairobi River. The concentrations of these heavy metals recorded in the water column in this study are higher than those obtained from previous studies of Nairobi River. Budambula and Mwachiro, (2005) reported mean concentration of Cu, Zn and Fe as all below detectable limits. Kithia and Ongwenyi, 1997 reported mean concentration Cu and Zn as 0.1 mg/L and 0.2 mg/L respectively. This increase in heavy metal content (Cu, Zn, and Cd) may be due to increased vehicular traffic, industries close to Nairobi River some of which may empty their effluents into the river and informal settlements along the river bank. The concentrations of Cu, Zn and Cd measured in the six sampling sites were not significant (p>0.05). The range of values of these metals (Cu = 18.35 ± 2.4 - 28.65 ± 4.3, Zn = 30.58 ± 5.5 - 85.64 ± 8.2, Cd = 21.58 ± 4.6 - 40.35 ± 3.2 mg/L) in water from the six sites are higher than the World Health Organization (WHO) limits (Cu = 1, Zn = 5, Cd = 0.05 mg/L) for drinking water (WHO 2010). The implication of this is that the water of Nairobi River is not fit for human consumption.

The concentration of Cu, Zn and Cd in soil among the sites was not significant (p>0.05). However, the mean concentrations in soil (Cu = 26.55 ± 4.8, Zn = 64.71 ± 7.3, Cd = 37.37 ± 5.2 mg/Kg) were higher than those found in water (Cu = 23.11 ± 4.5, Zn = 57.96 ± 6.8, Cd = 31.76 ± 5.5 mg/L) representing approximately 1.15 fold increase. This may be due to pre-concentration of the heavy metals in the soil and dilution effect in water due to water flow. This observation also agrees with the study of Oyewo, (1998) on Lagos Lagoon in Nigeria. Oyewo, (1998) opined that this observation is due to the fact that sediments acts as sinks of heavy metals derived from weathering as well as those from anthropogenic inputs. The biological significance of this observation is that flora and fauna especially benthic organisms which live on and forage on bottom soil will be exposed to greater risks of damage and or bioaccumulation.

The mean concentration of Cu (8.73 ± 0.5 mg/Kg), Zn (17.42 ± 2.4 mg/Kg) and Cd (4.19 ± 0.15) in A. hybridus among the sampling sites was not significantly different (p>0.05). The concentration of Cu, Zn and Cd in A.hybridus are within the normal range (Table 3-2) found in plants (Kabata-Pendias and Pendias, 1992).

3.2 Bio concentration of Cu, Zn and Cd by A. hybridus

The ability of the plants to take up heavy metals (BCF) was evaluated from the ratio of metal concentration in the plants and water. The mean BCF of A. hybridus was obtained as 8.44 ± 0.06. Table3 gives mean values of Bio Concentration Factor (BCF,) for each element. The mean BCF value of the elements in the plant decreases according to this sequence: Cd > Zn > Cu. This sequence (which is rather different from that of the mean
concentrations of elements in the plants) reflects the capacity of the plant species to accumulate elements independently from their concentration in the water, that is, the regulation capacity of the plants. The BCF of Cu, Cd and Zn ranged from 1.5 ± 0.02 for Cu to 12.41 ± 0.8 for Cd (Table 4). The BCF among the sampling sites was not significantly different (p>0.05). The ability of A. hybridus to absorb and concentrate these metals even when their values in water and soil are very small shows that A. hybridus could be a very good phytoremediant,

3.3 Heavy metal (Cu, Zn and Cd) pollution status of Nairobi River
The six sampling sites recorded varying concentration levels of the metals investigated in soil, water and the plants. The results show that site 4 and 5 are more polluted with zinc and cadmium compared to the other sites. The level of Cu at all sites does not differ significantly (p>0.5). Site 4 and 5 high pollution status may be due to effluent discharge, human and motor traffic, agricultural run-off coming through several drains along with washing and bathing activities by local inhabitants and cattle wading at peripheral villages in site 5 and 4. The high level of Zn and Cu might be due to agricultural run-off on sediments in the reservoir, carrying various Zn and Cu-based pesticides used in agricultural practices. This largely agrees with findings of Jones et al., (1991) in Lake Averno, and Siegel et al., (1994) in Ginka sub-basin, south of Lake Manzala. The results show that site 1 is also polluted with the heavy metals studied despite being the source of the river, which was used as a control. This could be attributed to the presence of flower farms in the area. The pollution may be due to the intensive use of agrochemicals containing heavy metals as active ingredients or contaminants. Zn showed the largest accumulation and can be considered as one of the major pollutants of Nairobi River.

4.0 Conclusion and Recommendation

4.1 Conclusion
The phytoremediation study conducted in this work provides significant information regarding suitability of A. hybridus as a bio-indicator for Cu, Zn and Cd heavy metal pollution. Bio-concentration factor (BCF) was determined. The BCF for A. hybridus was obtained as 8.44 ± 0.06. The plant was found to be a better accumulator of Cd (10.74 ± 1.5), followed by Zn (3.32 ± 0.4) then Cu (3.10 ± 0.3). The heavy metal concentration in water samples were in the range of 29.03 – 34.49, 22.37 – 23.87 and 53.36 – 62.52 mg/L for Cu, Cd and Zn respectively. In the digested soil samples metal concentration ranges were 25.81 – 27.31, 60.11 – 69.27 and 34.98 – 40.44 mg/Kg for Cu, Zn and Cd respectively. The concentration in the plant suggests that the plant had removed most of the heavy metals from the parent water. From this work, A. hybridus was found to be a reasonably good phytoremediant which can be used for effectively removing Cu, Zn and Cd polluted waters. A. hybridus is a suitable phytoremediant because it is able to accumulate Cu, Zn and Cd to a satisfactory degree. While the metal concentration in the water samples (37.61 ± 0.65 mg/L) were lower compared to that in soil (43.01 ± 0.30 mg/Kg) the plants were rich in heavy metal content. This is evidence of pre-concentration of heavy metals from water. The research hypothesis that plants growing along Nairobi River...
River do not offer significant clean-up potential by accumulation or uptake of heavy metals is rejected. Due to removal of heavy metals from polluted waters by plants, determination of heavy metal pollution in any water body by direct analysis of water samples may not be accurate because it will not reflect the real bio-available pollutant level in the water. This is because most of the heavy metal will be removed from the water to biota and sediment resident in the same water “use of bio-indicators like the plants e.g. A. hybridus would be more accurate”.

4.2 Recommendation
This study evaluated the use of A. hybridus for the remediation of heavy metal (Cu, Zn, and Cd) polluted aquatic ecosystem (Nairobi River). Thus the use of A. hybridus is recommended for phytoremediation of water polluted with Cu, Zn and Cd. Research related to this relatively new technology needs to be promoted, emphasized and expanded in developing countries like Kenya since it is low cost. Phytoremediation offers a viable solution to water pollution problems. This calls for multidisciplinary collaboration between universities, research institutes, and other interested parties to create teams to address questions like agronomic practices needed for successful establishment of vegetation; development or identification of locally available plant species for specific remediation requirements and fate and final disposal of biomass, particularly containing high concentration of metal.

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