

Assessment of heavy metals from the roots, barks and leaves of some selected medicinal plants (*Moringa oleifera* and *Azadirachta indica*) grown in Kashere metropolis

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Abstract

The increasing popularity and widespread use of medicinal plants as alternative medicine has sparked an interest in understanding their safety. Heavy metals have been identified as a risk to human health through the usage of medicinal plants. This study aimed to assess the level of heavy metals (Fe, Cu, Pb, Ni and Zn) in selected medicinal plants found in Kashere metropolis. Six different plants samples: MB, AB, CM3, AL, MR, and AR; representing: B = bark, L = leaf and R = root of both *Moringa oleifera* M, and *Azadirachta indica*, A; *Moringa oleifera* leave CM3; which are widely used for medicinal purpose were collected. Digested samples of these plants at standard concentrations were analyzed for heavy metal contents using Atomic Absorption Spectrophotometer. The result shows that CM3 had the highest concentration of Zn and Cu at 3.402 mg/kg and 0.3431 mg/kg respectively. Similarly, MR has the highest concentration of both Fe and Pb at 0.556 mg/kg and 0.9471 mg/kg respectively, while AB had the lowest concentration of Pb, Cu, and Fe. All the samples analyzed had concentration of heavy metals below the recommended permissible limits; as such the medicinal plants *Azadirachta indica* and *Moringa oleifera* are safe for use and consumable with regard to the five (5) heavy metals analyzed.

Keywords: Atomic Absorption Spectrophotometer, *Azadirachta indica*, Heavy metals, *Moringa oleifera*, Kashere metropolis.

1.0 Introduction

Man exists in an environment which is no longer friendly to him as a result of high anthropogenic pressure, which releases high concentration of toxic metals to the environment, and consequently causes negative effects on human and plant co-existence. Specifically, medicinal plants are those plants which are used for therapeutic or medicinal benefits and tend to be the viable source of many herbal products for therapeutic uses that thrives presently in Nigerian markets. The long standing use of medicinal uses is not only prevailing in Nigeria, as an estimate of about 80% of world population is said to rely on medicinal plants for their primary health care (Pirzada *et al.*, 2009). Most of these population dwell in the rural areas where conventional orthodox medical care are not sufficiently available, thereby making them much prone to use of such herbal plants (Esimone *et al.*, 2002, Omoseyindemi, 2003).

Nutrients have been viewed as food components that either cannot be synthesized in the body (for example, vitamin C) or whose synthesis requires a specific factor that may in certain circumstances be absent or inadequate (for example, some amino acids, fatty acids, and vitamins). There is now recognition that many other compounds of plant food, such as dietary fibre, flavonoids, sterols, phenolic acids, and glucosinolates, are associated with lower disease risk. Phytochemicals contained in plant foods especially vegetables have been linked to many positive effects on human health including; prevention and cure for coronary heart diseases, diabetes, high blood pressure, cataracts, degenerative diseases and obesity (Lui *et al.*, 2000). Vegetables are important source of biologically active substances such as oil, carbohydrates, minerals vitamins, dietary fiber, antioxidants and cholesterol-lowering compounds, as such regarded to be of importance to human health (Adenipekun *et al.*, 2010; Arzani *et al.*, 2007; Martin, 2005). Having established the health benefits of medicinal plants, it is obviously necessary to determine their level of heavy metal content as long time consumption of high doses can be toxic and destructive to human health.

In this work two medicinal plants that are widely known for their therapeutic and preventive purpose are being studied. They are *Azadirachta indica* (Neem) and *Moringa oleifera* (Horse-raddish tree), both of which are commonly and widely used for many ethno- medicinal purposes in Kashere. The aim of this research is to assess the level of some heavy metal from the root, bark and leaves of these plants. The study area is specifically important as major economic activities are coal mining and farming, both of which are practices that releases heavy metal effluents to the environment.

Azadirachta indica, a legendary anthelmintic, antifungal, antidiabetic, antibacterial, antiviral originated in Assam in northeast India, and Myanmar. It is common throughout the central dry zone and is locally called “sacred Tree”, “Heal All”, “Nature’s Drugstore”, “village pharmacy” and “Panacea for all diseases” (Ganguli, 2002). In the northern parts of Nigeria, *A. indica* is referred to as “*Bedi*, *Dogon yaro* or *Darbejiya*”. *Azadirachta indica* contains chlorophyll, calcium, phosphorus, iron, thiamine, riboflavin, nicotine, vitamin C, carotene, and oxalic acid. Highest concentrations of the active ingredients are found in the seed and oil, and to lesser amounts in the bark and the leaves. Each part of the plant has specific application. The bark, flower, fruit and root are used for analgesic, bile suppression, diabetes and diuretics respectively (Biswas *et al.*, 2002). The neem plant contains chemicals that help reduce blood sugar levels, heal ulcers in the digestive tract, and prevent plaque formation in the mouth (Bandyopadhyay *et al.*, 2004). It also has powerful pest controlling properties. More importantly, pesticides made from neem are non-toxic biodegradable compounds that do not contaminate the environment, thus much safer as compared to synthetic pesticides (Biswas *et al.*, 2002).

Moringa oleifera (“Zogale” in northern Nigeria) is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family *Moringaceae*. It is a fast growing, drought resistant tree commonly used for ethno medicinal uses in Philippines. In Africa and Indonesia; *Moringa oleifera* leaves are given to nursing mothers in the belief that they increase lactation in nursing mothers (Makkar *et al.*, 2007). The *Moringa* tree has been praised for its nutritional and medicinal properties, and many claims have been made. All parts of the tree including leaves, pods, bark, root has medicinal value, hence, it was called the miracle tree (Ijeh and Ejike, 2011).

Profound antimicrobial activities has been reported on extracts from leaves, roots, seeds, flowers and bark of *Moringa oleifera* against bacteria, yeast, dermatophytes and helminthes (Alam *et al.*, 2007; Renitta *et al.*, 2009; Caceres *et al.*, 1992). The plants have also shown moderate anti-inflammatory and anti-oxidant activity in rats (Singh *et al.*, 2009; Verma *et al.*, 2009; Ndiaye *et al.*, 2002; Medhi *et al.*, 1996), antitumor (Murakami *et al.*, 1998), anti-ulcer (Debnath *et al.*, 2007) and wound healing activities (Rathi *et al.*, 2006). Aqueous extract of the plant was assessed for nutrient composition and sensory evaluation. The results showed that the ash, crude fibre, fat and moisture contents of dry *Moringa oleifera* were 0.04, 0.00, 0.001 and 96.68%, respectively. In this same respect, protein and carbohydrate contents were 0.66 and 2.63%, each, while; iron, calcium, vitamin C and beta-carotene contents were 2.07, 33.35, 6.26 and 2.23 mg in the given order. Flavonoids and alkaloids contents were 0.20 and 0.07%, respectively (Madukwe *et al.*, 2013). These aforementioned biological activities of *Moringa oleifera* confirmed its medicinal uses and potentials.

Although, the periodic table contains 75% of metallic elements, those that specifically have specific densities greater than 5 gcm^{-3} or unique atomic weight and distinct toxicity are referred to as “heavy metals” (John, 2002; Berglund *et al.*, 2001). Such elements mainly include the transition metals, some metalloids, lanthanides and actinides. The metals are a cause of environmental pollution from sources like leaded petrol, industrial effluents and leaching of metal ion from the soil into lakes and rivers by rain wash-off from use of pesticides, herbicides and mining activities. Among these heavy metals, the most interesting ones considered for analytical study in this work are lead (Pb), iron (Fe), cobalt (Co), nickel (Ni) and zinc (Zn).

Lead poisoning or lead intoxication occurs when exposed to large concentration of the metal in the body beyond permissible level and is associated severe health effect (Grant, 2009; Guidotti *et al.*, 2007). No safe threshold for lead exposure has been discovered, and that there is no known amount of lead that is too small to cause the body harm (Guidotti *et al.*, 2007). Iron is the most abundant and relatively highest environmentally benign of the heavy metals. Its safe nature could be predicted as it forms the central metal in the hem that constitutes hemoglobin and essential micronutrients for human growth, development and maintenance of the immune system. Iron is needed for psychomotor development, maintenance of physical activity, work capacity and resistance to infection (Stoltzfis, 2001). On the other hand, nickel is carcinogenic and teratogenic. Because it is locked away in the earth’s iron-nickel molten core, which is 10% nickel, the total amount of nickel dissolved in the sea has been calculated to be around 8 billion tons. Organic matter has a strong ability to absorb the metal. This is why coal and oil contain considerable amounts. Nickel is a compound that occurs in the environment only at very low levels. The uptake boost when people eat large quantities of vegetables from polluted soils. Plants are known to accumulate nickel and as a result the uptake from vegetables will be eminent (Leuntech, 1998). Copper is a very common substance that occurs naturally in the environment and spreads through the environment through natural phenomena. The absorption of copper is necessary, as it is a trace element that is essential for human health. Although humans can handle proportionally large concentrations of copper, too much copper can still cause eminent health problems (Lenntech, 1998). Many food stuffs contain certain concentrations of Zinc. Drinking water also contains certain amount of zinc, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the zinc amounts in drinking water to reach levels that can be detrimental to health. As a trace element, zinc is essential for human health. Industrial sewage contains a large amount of zinc, and its presence in such waters can interrupt the activity in soils, as it negatively influences the activity of micro-organisms and earth worms.

Despite all the afore mentioned importance of both *Azadirachta indica* and *M. oleifera*, the biological activities studied and the toxic and harmful potentials of the heavy metals that could be accumulated

in the leaves and stark of these plants; little or no work is available on the analytical assessment of the heavy metal contents of these plants. To the best of our knowledge, the assessment of the heavy metals concentration in the root, leaves and stark of both *Azadirachta indica* and *Moringa oleifera* grown in Kashere (Gombe State) and actively used for their ethno medicinal and food potential is therefore novel. Henceforth, herein we report the assessment of level of Pb, Cu, Zn, Fe and Ni in the sample parts of these medicinal plants. The result of this research can be helpful to the dwellers of Kashere in revealing the risk or none involved in the consumption of such plants.

2. Materials and method

All glass ware, mortar, pestle and other surfaces are thoroughly washed and dried in an oven at 120 °C. Analytic balance (JF/JTA JA 5003 series, range: 0 – 500 g) was used for all weighing and standard mass/weight unit or volume units are used. Plant parts used were obtained fresh from local farms, properly identified and specimen sample labeled and kept in the herbarium. All chemicals used are of analytical grade purchased from Sigma-Aldrich or Fluka and used as obtained. Atomic absorption measurements were made using 21OVGP Flame AAS cathode lamps (Abubakar Tafawa Balewa University, Bauchi, Nigeria). Air-acetylene flame was used to determine all the elements prepared samples.

2.1 Digestion of samples

10 grams of plants samples were ashed in a muffle furnace at 400 – 450 °C. The residues obtained were then dissolved in HNO₃ (15 cm³). Afterward, HCl (5 cm³) and few drops of 30% H₂O₂ were added. The undissolved carbon was filtered off and the filtrate diluted to 100 cm³. Concentrations of heavy metals were then determined in the samples using the diluted filtrate. The afforded solutions were analyzed for Pb, Fe, Ni, Cu, and Zn using Atomic Absorption Spectrometer (AAS) (AOAC, 2000).

3. Results and discussion

3.1. Samples and sampling

The two (2) plant samples; *Moringa oleifera* and *Azadirachta indica* (leaves, bark and roots) were obtained from farms within Kashere metropolis and transferred to the laboratory. The samples were thoroughly washed with distilled water to free the sample from earth and dirt then air dried. They were then pounded to fine granules individually using mortar and pestle and finally sieved. After which the samples were then stored in different plastic bags and labeled accordingly as presented in table 1:

Table 1: Coding of plants samples

Name of sample	Code
<i>Moringa oleifera</i> bark	MB
<i>Moringa oleifera</i> leave	CM3
<i>Moringa oleifera</i> root	MR
<i>Azadirachta indica</i> bark	AB
<i>Azadirachta indica</i> leave	AL
<i>Azadirachta indica</i> root	AR

The concentration of some heavy metals such Pb, Fe, Ni, Cu and Zn was determined from the root, bark and leaf of the two medicinal plants. Dry ashing of the plants materials and subsequent use of Atomic Absorption Spectrometer (AAS), were the analytical methodologies used. All analyses were measured in triplicate and results presented as Mean concentration (mg/kg) and standard deviation (Table 2). An illustrative comparism of each heavy metal in the samples was also presented in Figures 1 - 4. The fact that Ni concentrations in all the test samples were below detection limit (Table 2), no pictorial representation of its mean concentration was presented.

Table 2: Mean concentration alongside the probable standard deviation (mg/kg $\pm \sigma$) of heavy metals in the plants samples

Entry No.	Sample code	Concentration of heavy metals with standard deviation (mg/kg $\pm \sigma$)				
		Pb	Fe	Ni	Cu	Zn
1	MB	0.1641 \pm 0.1600	26.50 \pm 0.017	β	0.1407 \pm 0.0155	2.397 \pm 0.005
2	AB	0.5558 \pm 0.0317	35.40 \pm 0.011	β	0.1975 \pm 0.0305	2.109 \pm 0.004
3	CM3	0.2792 \pm 0.0346	27.92 \pm 0.003	β	0.3431 \pm 0.0093	3.402 \pm 0.011
4	AL	0.4790 \pm 0.0273	28.20 \pm 0.014	β	0.2647 \pm 0.0286	2.815 \pm 0.013
5	MR	0.9471 \pm 0.0173	55.60 \pm 0.012	β	0.1762 \pm 0.0230	3.225 \pm 0.022
6	AR	0.3862 \pm 0.0128	28.00 \pm 0.001	β	0.2909 \pm 0.0252	3.164 \pm 0.012

Note: β = below detectable limits

Plants absorbs metal ion from the soil through the roots. The uptake of these ions is influenced by various factors including type of plant, nature of soil, climate and practices. The concentration of heavy metals is not uniformly distributed in the plant. In general, the roots contain the highest levels of heavy metals, followed by vegetative tissue which has higher concentration than seeds or grains (Cui *et al.*, 2004).

The mean concentration of lead Pb, in the *Moringa oleifera* samples was found within the range of 0.1641 – 0.9471 mg/kg, while that of *Azadirachta indica* within the range of 0.3862 – 0.5558 mg/kg (Table 2). In this same vein, lowest and highest mean concentration values of the Pb were observed in *Moringa oleifera* bark (MB) and root (MR) respectively (Figure 1). The result showed that the plant samples accumulated different amount of Pb in the three

parts surveyed. The main sources of lead contamination are: lead mines, bush burning, combustion and industrial sewage (Ahmad *et al.*, 2006). For this particular study area, the level of Pb could also be attributed to iron bending and welding of metals which is common practice in the area. Pb is considered highly hazardous for plants, animals and particularly for micro-organisms. Exposure to Pb is of concern mainly because of its acute toxicity even at trace levels. Numerous studies have revealed that it can adversely affect the central and peripheral nervous system (Bellinger *et al.*, 1992). In this study, the amount of Pb found in the samples fall below the national and international guidelines for metals in food and vegetables (Tables 2 and 3), this makes the plants samples to be suitable for consumption with regard to lead.

Iron Fe, was found to be the dominant element in the root of *Moringa oleifera* with highest concentration of 55.60 mg/kg to the lowest concentration of 26.50 mg/kg. Range of 35.40 – 28.00 mg/kg for Fe concentration was also observed in the case of *Azadirachta Indica*. This high concentration of iron is to great advantage as both plants and animals require Fe for proper growth and development. In human it is vital for metabolic process such as DNA synthesis and oxygen transport to cells (Ogbe *et al.*, 2012). Daily intake of iron supplements reduces the risk of anemia; and its deficiency can cause various types of diseases (Upadhyay *et al.*, 2001). Fe concentration from all the six samples range from 55.60 mg/kg to 26.50 mg/kg (Figure 2), which is within the recommended permissible limits for WHO/FAO (Table 3). Therefore, this makes the plants samples suitable for consumption with regard to Fe (FAO/WHO, 2007).

Nickel Ni, was not detected from the six collected sample. This may be as a result of passive ability of Ni to react (uncreative, because of a thin inert layer of oxide) when treated with nitric acid just as Fe (during digestion). It might also be due to absence of emission from vehicle engines that uses gasoline, which is in combination with Ni containing compounds as additive and corrosion of nickel from vehicle parts (Al-shayeb & Seaward, 2001).

Copper Cu, concentration is fairly high in the leaves of *M. Oleifera*. Copper, although an essential plants micro nutrient, is just required in trace amounts in plants (Cui *et al.*, 2004). The most common sources for Cu in the environment are: Pesticides, Fertilizers, industries and sewage sludge. The concentration of copper in all the six samples were relatively low ranging from the highest value of 0.3431 mg/kg to the lowest value of 0.1407 mg/kg in

Moringa oleifera. In *Azadirachta indica*, the range of 0.2909–0.1975 mg/kg was recorded for concentration of Cu. The concentration is relatively low compare to the standards given by WHO/FAO (Table 3 and Figure 3). This ranged of copper concentration is considered non-toxic to plants and animals because it is lower than the critical threshold levels which ranged from 15 - 200 mg/kg (Alloway, 1995).

The concentration of zinc Zn, in the six plants samples is relatively high, ranging from the highest value of 3.402 mg/kg to the lowest value of 2.397 mg/kg in *Moringa oleifera* and to the highest value of 3.164 mg/kg to the lowest value of 2.109 mg/kg in *Azadirachta indica*. Zinc is an essential element for humans (COMA, 1991). At present it is one of the essential and enzymatic elements for plants, animals and human beings. Most plant species were found to be tolerant to higher Zn concentration. The general sources of Zn contamination are: agrochemicals, burning of fossil fuels and sewages. Due to its importance; Zn is present in blood and about 85% of it combines with protein for after absorption and its turnover is rapid in pancreas. Deficiency of Zn causes hypersonmia, hypogensia or coma (FAO/WHO, 2007).

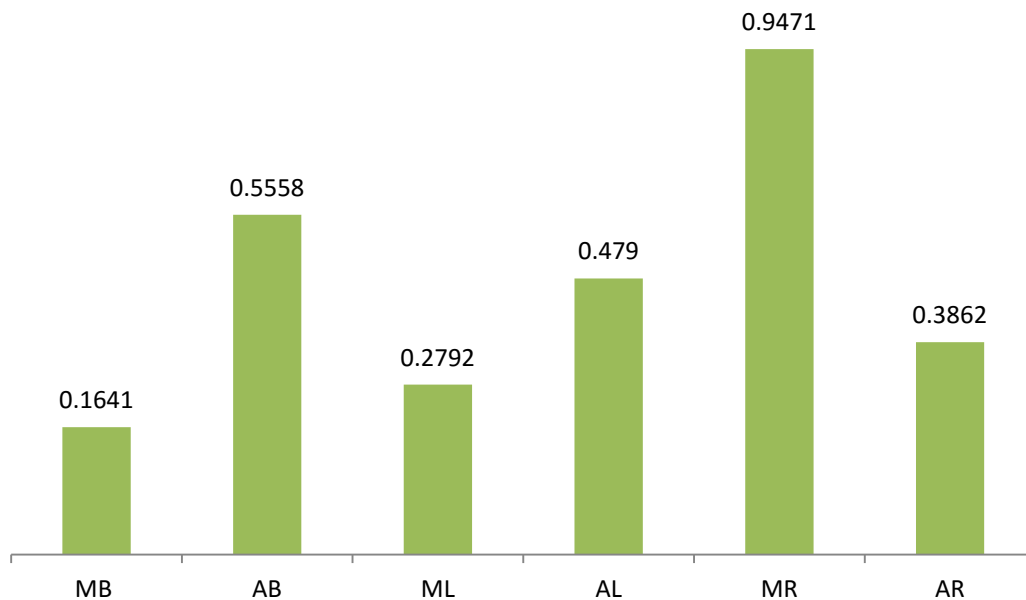


Fig. 1: Mean concentration of Pb (mg/kg)

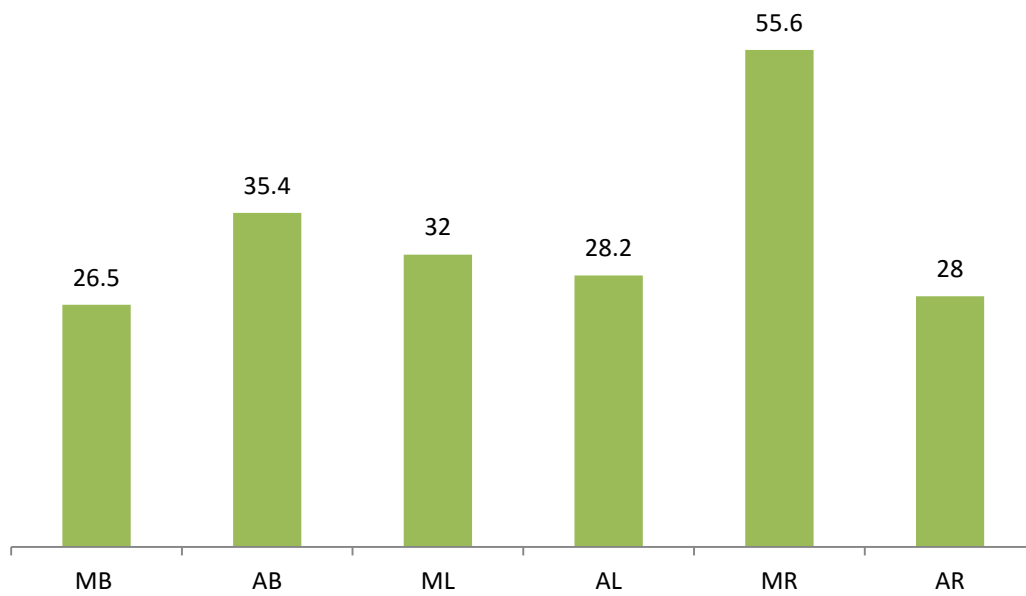


Fig. 2: Mean concentration of Fe (mg/kg)

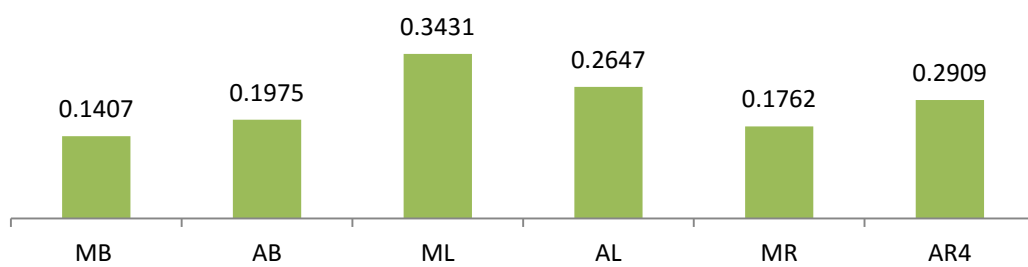


Fig. 3: Mean concentration of Cu (mg/kg)



Fig. 4: Mean concentration of Zn (mg/kg)

Table 3: Comparison between normal, toxic and permissible concentration of heavy metals in plants (Kabata-Pendias, 2011)

Entry No.	Metal	Concentration (mg/kg)		
		Normal	Toxic	Permissible range
1	Cu	3–15	20	2–5
2	Pb	1–5	20	0.50–30
3	Zn	15–150	200	20–100
4	Fe	50–250	>500	400–500
5	Ni	0.1–5	30	0.02–50

4. Conclusion

Six different parts of plants samples use for medicinal purpose (MB, AB, CM3, AL, MR, and AR) were collected within Kashere metropolis. The samples were acid digested and analyzed for heavy metals using Atomic Absorption Spectrophotometer. The concentrations of the heavy metals (Lead, Zinc, Copper, iron and Nickel) were determined and the order of concentration is Fe > Zn > Pb > Cu, while nickel is way beyond detection limits. The results obtained shows that medicinal plants from Kashere metropolis contains heavy metals (Pb, Ni, Cu, Zn and Fe) which does not exceeds the recommended permissible range of WHO. It is also found that the concentration of heavy metals is

higher in the root and leaves of the plants' samples. This could be attributed to the fact that absorption of nutrient and photosynthesis activities occurs in the root and leaves respectively.

It is of paramount importance for heavy metal content of medicinal plant used for various types of ailments to be checked, in order to make it safe for human consumption. This study provides useful reference data for the standardization of medicinal plant materials. It will however be of economic value to nation's economic sector cultivation and medicinal potential of these plants are judiciously utilized. Nigeria is one of such countries blessed with numerous resources and compatible land for growing these important trees. Efficient management of their production could assist in fighting malnutrition, medicine, and can be used as an anticoagulant in water treatment plant.

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