**ICP-MS Analysis of Elemental Composition in Soils and Forage Plants Irrigated with Sewage water and Bore well water**

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**Abstract**

Ensuring sufficient nutrition for livestock is crucial for maximizing livestock productivity besides bridging the widening gaps between demand and supply of green fodder. The cumbu napier hybrid grass, known for its high biomass yield per unit area and wider adaptability has played a vital role in managing the green fodder requirement to a substantial extent is the focus of the current study. Present research aims to explore the impact of sewage waste water on the growth of Cumbu Napier hybrid grass. The findings revealed that elevated levels of heavy metals viz., 37.77 ppm of lead and 10.46 ppm of tin accumulation in Cumbu Napier hybrid grass grown in soil irrigated with sewage water was recorded as compared to those irrigated with borewell water where 7.73 ppm of lead and 1.92 ppm of tin were registered. Hence, the results indicated that recycling the sewage water for high water demanding forage crops such as cumbu napier hybrid grass found to accumulate more heavy metals than bore well water. The order of mean concentrations of heavy metals in the soil so obtained was Sn>Sb>As>Pb>Cd.

**Key words: Sewage water, borewell water, elemental analysis, ICPMS, Microwave digestion technique.**

**Introduction**

In India, fodder is cultivated in approximately 9.13 million hectares, representing about 4% of the nation's total cultivable land. Freshwater is vital for sustaining agriculture, industry, and human life (Abdul *et al.,* 2012). Yet, when it comes to producing cattle feed and fodder, the practical and effective solution would be utilizing sewage water or domestic wastewater which is dosed without proper utility although water scarcity is alarming in many part of our country. However, a notable challenge in utilisation of waste water arises due to the presence of heavy metal contamination. Current research work endeavours to explore and address a crucial nexus within the agricultural landscape - the intricate relationship between water scarcity, fodder cultivation, and livestock sustenance. Focusing on India, a country recognized for hosting the world's largest livestock population and serving as a vital hub for the rural economy, this study delves into the implications of water scarcity on the availability of irrigated fodder crops crucial for sustaining livestock productivity. To meet the current level of livestock production and its annual growth in cultivation of perennial fodder varieties which can yield higher biomass per unit area is the immediate solution (Babu *et al.,* 2014). By unravelling the complexities of this dynamic interplay, the research aims to contribute valuable insights into the sustainable management of water resources, fodder cultivation practices, and the overall productivity of livestock systems in the context of evolving global challenges.

However, the burgeoning demand for water in agriculture, intensified by a high population, has resulted in a scarcity of water for irrigation. In response to this pressing issue, a sustainable solution involves harnessing alternative water sources, such as sewage water or domestic wastewater for soil irrigation, particularly in the cultivation of fodder crops. This practice not only alleviates water shortages but also aligns with principles of water conservation and responsible resource management, offering a potential avenue for sustainable agriculture in regions grappling with water constraints due to burgeoning populations.

The current research focuses on assessing the nutritional composition of sewage water and borewell water, aiming to understand how these distinct water sources influence the soil properties, subsequently, the nutritional composition of the fodder crops cultivated in that soil. Inductively Coupled Plasma Mass Spectrometry (ICPMS) stands out as the most potent technique among atomic spectrometry methods for identifying trace and ultra-trace elements at levels ranging from parts per billion (ppb) to parts per million (ppm). This is attributed to its exceptional sensitivity, low detection limits, and the ability to detect multiple elements simultaneously(Hill, 2007 &Hansen and Pergantis, 2007). Through a meticulous comparative study, the research extends beyond nutrient analysis to include an examination of heavy metal levels, providing a comprehensive understanding of the potential environmental and agricultural implications associated with using these water sources for irrigation. This multifaceted approach contributes valuable insights into sustainable water management practices and the overall safety of fodder production and feeding to livestock.

**Materials and methods**

The present study was carried out at the Department of Forage crops, Tamil Nadu Agricultural University, Coimbatore. This study aims to analyse heavy metals in soils and cumbu napier grass grown under sewage water and bore well water. The present study is focused on analyzing the suitability of sewage water for fodder production. Thus, six different kinds of samples were taken for analysis, *viz*., sewage water, bore well water, soil irrigated with sewage water, soil irrigated with bore well water, fodder plants grown under sewage water, and fodder plants grown under bore well water. After the pre-processing of samples and   proper   microwave digestion, the samples have been analyzed for elements. The fodder crop used in the present study is the cumbu napier or bajra napier hybrid grass (CO (BN) 5) which is of interspecific origin. Although this fodder crop yields highest biomass (365 t/ha) among available various fodder resources, it demands high water (1800 – 1900 mm) throughout the year for its cultivation.

**Chemicals and Reagents**

Multi element Aqueous CRM which is a mixture of 30 elements i.e., Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Si, Sn, Ti, Tl, V, Zn 100 µg/ml in 5% HNO3was procured from VHG Labs, Manchester, NH , USA. Nitric acid (HNO3), 67-70%, for trace metal analysis was procured from Fisher Chemicals, India. HPLC grade water was used for the preparation of all samples and standards. All lab accessories used were of “A” grade and duly calibrated. Calibrated micropipettes were used with the range of 2µl to 1000µl.

**Instrumentation**

Inductively Coupled Plasma-Mass Spectrometry [ICPMS], Thermo Scientific™ iCAP™ RQ, equipped with micro mist borosilicate glass nebulizer; quartz cyclonic spray chamber; ICP torch, nickel sampler cone and skimmer cone, Quadrapole mass analyser and mass spectrometry detector. All the samples were analysed in Kinetic Energy Discrimination (He KED) mode using pure He as the collision gas in the collision/reaction cell (CRC) under optimized auto tune conditions of the equipment directly from Quality control with Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software. To automate the sampling process, an ASX‑560Auto sampler (Omaha, NE, USA) was used. Sample digestion was performed through a closed-vessel microwave digestion system – Multiwave GO (Anton Paar) with a multi wave pro rotor, temperature, and pressure sensor, provided with an auto pressure vent PTFE vessels.

**Preparation of Calibration Standard solutions**

Accurately 1ml of mixed standard reference solution was pipetted into a 100 ml volumetric flask diluted to the volume to 100 ml using HPLC grade water. This was taken as the stock solution used for the preparation of calibration standard solutions and was stored under suitable conditions. Appropriate aliquots were taken and further diluted with 5% nitric acid in HPLC grade water so as to give a series of calibration standard solutions having the concentration range of 1.0, 20.0, 50.0 and 100.0 µg/l.

**Preparation of Sample solutions**

Approximately 0.20 ±0.01g, accurately weighed, dried homogenized powder samples (already milled with Teflon mortar to avoid metal contamination) of each soil and plant leaf samples were taken in 6.0 ml of concentrated ultra-pure HNO3 in a tightly closed PTFE vessel and digested in a microwave digester separately. The digestion was carried out in three steps with a constant microwave power, the program was set to increase the temperature 160°C in 10 mins and held at that for 5 mins and cooled down the digester at room temperature and diluted the digested samples with HPLC grade water up to 50 ml. Three replicate samples were prepared for each sample for analysis.

**ICP-MS operating conditions**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| RF power (W) | **1500** |  | Nebuliser pump (rpm) | **40** |
| Plasma gas flow rate (L min−1) | **14** | Extract lens (V) | **1.5** |
| Auxiliary gas flow rate (L min−1) | **0.8** | Pirani pressure (mbar) | **1.81** |
| Carrier gas flow rate (L min−1) | **1** | Penning pressure (mbar) | **3.05x10-7** |
| Spray chamber 𝑇 (∘C) | **2** | Plasma exhaust (mbar) | **0.45-0.56** |
| Sample depth (mm) | **8.6** | Number of replicates | **3** |

**3. Results and discussion**

Ensuring the proper levels of essential elements is important for the health of the livestock. The elements are integral part of an animal's diet and are commonly included in feed production. A deficiency in crucial elements such as magnesium, phosphorus, potassium, boron, sodium, cobalt, copper, iron, manganese, zinc and calcium can lead to severe adverse physical effects and, in some cases, even result in death (Georgievskii*et al.,* 1992 & Suttle, 2010). The results obtained by the He collision method in ICPMS with MDS have been tabulated in Table 1. It provides detailed information on micro and macro nutrients derived from water to soil to plants and the results are presented in parts per million. Bore well water recorded 69.02ppm of sodium, 25.17ppm of magnesium, 2.441ppm of potassium, 34.68ppm of calcium. Additionally, there are trace amounts of 0.01-0.1ppm for boron, phosphorus, manganese, iron, copper, zinc, and molybdenum, while cobalt is not detected, registering at zero levels.

In sewage water, all these essential nutrients were in elevated quantities with percentages varying from 10% (Magnesium) to 90% (Zinc) than the bore well water due to contamination from different sources. In soil, the nutrients in the soil irrigated with sewage water are high (4% to 35%) except phosphorus, copper and zinc. In soil, the nutrients in the soil irrigated with sewage water were noted high percentages except phosphorus, copper and zinc. The soil irrigated with sewage water exhibits comparatively high levels of major toxic heavy metals, including tin, chromium, nickel, and lead. Fodder plants irrigated with sewage water also show a higher accumulation of heavy metals, as indicated by concentrations such as 10.46 ppm of tin and 37.77 ppm of lead in the samples depicted in Figure 1.

It is understood from the present study that elemental composition of the sewage water-irrigated soil influences the nutrient composition of fodder plants grown in that particular soil. The contamination of soil with toxic trace metals and their accumulation is a major concern for fodder production which exerts adverse effects on crop growth (Nagajyothi*et al.,* 2010). This might be due to the accumulation of heavy metals in soil including vanadium, chromium, nickel, mercury, cadmium, lead, and arsenic and none of which serve any beneficial nutrition to the crops and are toxic even at low concentrations. Livestock is especially vulnerable to the dangers posed by these toxic elements, leading to extensive research to establish the levels at which toxic symptoms manifest in different animal species (Bampidis*et al.,* 2013). The order of mean concentrations of heavy metals in the soil so obtained was Sn>Sb>As>Pb>Cd. In the comparison between soil irrigated with sewage water and soil irrigated with borewell water, an increase of 13-16% has been observed for vanadium, chromium, nickel, arsenic, and silver concentration in the sewage water-irrigated soil. However, Tin and lead exhibited even higher percentages when compared to all the other heavy metals, attributable to contamination from various surfaces exhibited in Figure 2. Among soil pollutant, lead (Pb) is one of the toxic metal pollutants (Shahid*et al.,* 2011). Lead negatively affects various aspects of plant growth, seed germination, seedling development, root elongation, transpiration, chlorophyll production, lamellar organization in chloroplasts, and cell division (Gupta *et al.,* 2010).However, the heavy metals are taken up by fodder plants as they grow in the soil. Alternate approach of recycling of sewage water, crop rotation and interchange of sewage and bore well water irrigation at certain period will augment the utility of sewage water during crucial condition of water scarcity.

**Impact of nutrients on crop growth**

It includes the two categories of macro and micro nutrients. We have analyzed the levels of macronutrients (Ca, Mg. P and K) and micronutrients (B, Na, Mn, Fe, Co, Cu, Zn, and Mo) in the different categories of water, soil, and fodder plants. The results obtained in the present study and their impacts were well discussed below for comprehensive understanding. These macro and micro nutrients are highly essential for plants to survive, grow, develop and reproduce. Among macro nutrients, nitrogen, phosphorus and potassium are required in large quantities while rest of the macro nutrients are required in moderate quantities to the plants. Micro nutrients are required in small quantities.

Phosphorus is essential for root development, production of flowering, fruiting, transportation of energy and resistant to diseases. Calcium is mainly involved in the cell division and regulates hormonal activity. Potassium involves in the maintenance of osmotic potential of the cell, transfer of sugar and starch and act as an iron carrier in oxidation-reduction process. Magnesium is a vital component of chlorophyll which produces green colour leaves, involves in the production of carbohydrates, oil, fats and vitamins and regulated the uptake of nutrients. Iron involves in the protein synthesis, absorption of other nutrients in plants and oxygen carrier during respiration. Zinc influences the production of growth hormone like auxin and promotes the reproduction process. Manganese is essential for movement of iron, photosynthesis and carbohydrate production. Cobalt plays a vital role in plant metabolism. Copper can neutralize the soil which is at harmful conditions, formation of essential compounds derived from protein and amino acids. Molybdenum is involved in the fixation of atmospheric nitrogen by increases the efficiency of legume crops and synthesis of vitamin C. Boron increases the absorption rate of nitrogen in plants, regulates potassium/calcium ratio and essential for formation root nodules (Agritech Portal, 2021). These elements are considered as essential nutrients if it is at the optimum level and as toxic if it get accumulates beyond the optimum level.

**Impact of heavy metals on plants**

The heavy metals include copper, vanadium, chromium, nickel, arsenic, silver, cadmium, tin, antimony and lead. Copper is also produces toxic effect of plant growth retardation, leaf chlorosis and production of reactive oxygen species (Stadtman and Oliver, 1991). Cadmium reduces the absorption rate of nitrate by inhibiting the nitrate reductase activity and also reduces the activity of enzymes involved in CO2 fixation (Filippis and Ziegler, 1993). Chromium affects photosynthesis in plants by creating snag in the CO2 fixation, transport of electrons and activity of enzymes (Clijsters andVan Assche, 1985). Lead is the most abundant toxic elements available in soil (Asati*et al.,* 2016). It creates negative effects on plant growth, morphology and photosynthesis (Juwarkar and Shende, 1986). Excess nickel produces necrosis, chlorosis and affects calvin cycle in plants (Sheoran*et al,* 1990). Arsenic reduces fruit yield and leaf weight in tomato (Barrachina*et al.,* 1995). In the present investigation, the mean percentages were determined for Tin, Lead, Cadmium, Antimony, Copper, Chromium, Nickel, Vanadium and Arsenic in cumbu napier grass irrigated with sewage water, with the order being Tin > Lead > Cadmium > Antimony > Arsenic.

**Conclusion**

             The study revealed a notable variation in the elemental composition of sewage water and bore well water, emphasizing the complexity of their impact on irrigated soil and the subsequent elemental composition of fodder crops. Irrigated plants exposed to untreated sewage water demonstrate elevated concentrations of heavy metals, emphasizing the necessity for water treatment prior to its utilization in irrigation practices. By recycling the sewage water, minimizing the potential adverse affects on soil health and the safety of fodder production systems, fostering a more sustainable and resilient agricultural ecosystem with minimum detrimental effect to the live stocks feed with the fodder resources produced. Water scarcity has been mitigated through the utilization of treated sewage water, resulting in increased yields of fodder crops. This underscores the positive impact of employing treated wastewater in agricultural practices to enhance productivity while addressing water resource challenges.

**Table.1 Analysis of elements of water, soil and forage crop samples**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Borewell water (in ppm)** | **Sewage water  (in ppm)** | **Soil irrigated with borewell water  (in ppm)** | **Soil irrigated with sewage water   (in ppm)** | **Fodder plant**  **irrigated with borewell water (in ppm)** | **Fodder plant irrigated with sewage water  (in ppm)** |
| **Boron** | 0.143 | 0.384 | 4.254 | 4.891 | 11.015 | 22.464 |
| **Sodium** | 69.02 | 171.15 | 85.559 | 132.95 | 11.433 | 24.598 |
| **Magnesium** | 25.178 | 28.099 | 2153.69 | 2340.02 | 1760.3 | 2390.25 |
| **Aluminium** | 0.184 | 1.39 | 4894.12 | 6402.58 | 13.607 | 17.271 |
| **Phosphorus** | 0.045 | 1.888 | 238.846 | 141.321 | 311.84 | 1291.18 |
| **Potassium** | 2.441 | 7.172 | 802.192 | 839.51 | 3305.79 | 6110.46 |
| **Calcium** | 34.68 | 45.982 | 3623.17 | 5080.21 | 1772.73 | 2485.53 |
| **Manganese** | 0.005 | 0.052 | 136.9 | 162.023 | 37.973 | 55.06 |
| **Iron** | 0.024 | 0.116 | 523.975 | 576.855 | 3.452 | 3.833 |
| **Cobalt** | Nil | 0.001 | 4.317 | 4.888 | 0.136 | 0.376 |
| **Copper** | 0.015 | 0.235 | 7.872 | 6.993 | 2.718 | 3.193 |
| **Zinc** | 0.008 | 0.085 | 17.76 | 14.169 | 10.072 | 21.671 |
| **Molybdenum** | 0.001 | 0.007 | 0.041 | 0.044 | 0.380 | 0.524 |
| **Titanium** | 0.142 | 0.211 | 223.965 | 242.33 | 5.363 | 7.872 |
| **Vanadium** | 0.027 | 0.06 | 15.741 | 18.421 | 0.066 | 0.089 |
| **Chromium** | Nil | 0.003 | 5.839 | 6.948 | 0.432 | 0.494 |
| **Nickel** | 0.001 | 0.006 | 6.713 | 7.846 | 0.512 | 0.589 |
| **Arsenic** | Nil | 0.001 | 0.796 | 0.95 | 0.011 | 0.021 |
| **Silver** | Nil | 0.002 | 0.130 | 0.151 | 0.016 | 0.016 |
| **Cadmium** | Nil | Nil | 0.025 | 0.029 | 0.008 | 0.034 |
| **Tin** | Nil | 0.003 | 0.321 | 0.574 | 1.925 | 10.463 |
| **Antimony** | Nil | Nil | 0.060 | 0.078 | 0.055 | 0.126 |
| **Cesium** | Nil | Nil | 0.409 | 0.336 | 0.005 | 0.003 |
| **Lead** | 0.003 | 0.016 | 3.271 | 3.851 | 7.739 | 37.778 |

**Figure 1.Comparative analysis of heavy metal levels in soil and fodder plants irrigated with sewage water and bore well water**

**Figure 2. Percentage of heavy metal accumulation in the fodder plants grown with untreated sewage water**

**References**

* Abdul, R. M., Mutnuri, L., Dattatreya, P. J., and Mohan, D. A. 2012. Assessment of drinking water quality using ICP-MS and microbiological methods in the Bholakpur area, Hyderabad, India. *Environ. Monit. Assess.,* **184**:1581–1592. (doi.org/10.1007/s10661-011-2062-2)
* Agritech portal, 2021 (https:// agritech. tnau.ac.in/ agriculture/ agri\_ min\_ nutri\_ essential elements.html)
* Asati, A., Pichhode, M. and Nikhil, K. 2016. Effect of Heavy Metals on Plants: An Overview. *International Journal of Application or Innovation Engineering & Management*., **5(3):** 56-66. (doi.org/ 10.13140/RG.2.2.27583.87204)
* Babu, C., Iyanar, K. and Kalamani, A. 2014. High green fodder yielding new grass varieties. *Electronic Journal of Plant Breeding.,* **5(2):** 220-229.
* Bampidis, V. A., Nistor, E. and Nitas, D. 2013. Arsenic, cadmium, lead and mercury as undesirable substances in animal feeds. *Sci. Papers Anim. Sci. Biotechnol.,* **46(1)**:17–22.
* Barrachina, C., Carbonell, F. B. and Beneyto, J. M.1995. Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenite on plant growth and yield. *Journal of Plant Nutrition.,***18(6):**1237– 1250.(doi.org/10.1080/01904169709365344).
* Clijsters, H and Van Assche, F., 1985.Inhibition of photosynthesis by heavy metals. *Photosynth Res*., **7:**31–40. (doi.org/ 10.13140/RG.2.2.27583.87204)
* Filippis, L. F and Ziegler, H. 1993. Effect of sublethal concentrations of zinc, cadmium and mercury on the photosynthetic carbon reduction cycle of Euglena. *J Plant Physiol.,* **142:167**–172. (doi.org/10.1016/S0176-1617 (11)80958-2).
* Georgievskii, V.I., Annenkov, B.N., and Samokhin, V.T. 1982. Mineral Nutrition of Animals, 1st Ed., Butterworth-Heinemann, Oxford, United Kingdom.
* Gupta, D. K., Huang, H.G., Yang, X. E., Razafindrabe, B. H. N., and Inouhe, M. 2010. The detoxification of lead in Sedum alfredii H. is not related to phytochelatins but the glutathione. *J Hazard Mater.,* [**177**](https://doi.org/10.1016/j.jhazmat.2009.12.052)**(**[**1-3**](https://doi.org/10.1016/j.jhazmat.2009.12.052)**):** 437–444. (doi.org/10.1016/j.jhazmat.2009.12.052).
* Hansen, H. R. and Pergantis, S. A. 2007. Identification of Sb(V)-complexes in biological and food matrices and their stibine formation efficiency during hydride generation with ICP-MS detection. *Anal. Chem.,* **79(14):** 5304–5311. (doi.org/ 10.1021/ac070130r.).
* Hill, S.J. 2007.Inductively Coupled Plasma Spectrometry and Its Applications. Blackwell Publishing., 61–73.
* Juwarkar, A.S., Shende, G. B.1986. Interaction of Cd-Pb effect on growth yield and content of Cd, Pb in barley. *Ind J Environ Heal*., **28:**235–243.
* Nagajyoti, P.C., Lee, K.D., and Sreekanth, T.V.M. 2010. Heavy metals, occurrence and toxicity for plants: A review. *Environ ChemLett*., [***8***](https://doi.org/10.1007/s10311-010-0297-8):199–216. (doi.org/ 10.1007/s10311-010-0297-8).
* Shahid, M., Pinelli, E., Pourrut, B., Silvestre, J., and Dumat, C. 2011. Lead-induced genotoxicity to Viciafaba L. roots in relation with metal cell uptake and initial speciation. *Ecotoxicol Environ Saf.*, 74(1): 78–84. (doi.org/ 10.1016/j.ecoenv.2010.08.037).

Sheoran, S., Singal, H. R. and Singh, R.1990. Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeon pea (Cajanus cajan L.). *Photosynthesis Research.,***23(3):** 345–351. (doi.org/ 10.1007/BF00034865).

* Stadtman, E. R., Oliver, C. N., 1991. Metal catalyzed oxidation of proteins. Physiological consequences. *J Biol Chem*., **266**:2005–2008.
* Suttle, N.F. 2010. Mineral Nutrition of Livestock, 4th Ed., MPG Books Group, Bodmin, United Kingdom.