



Effect of heavy metals on the features of medicinal plants at a quarrying site, in Ishi-Agu Ebonyi state Nigeria

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Abstract

Objective: This work intended to determine the concentration of heavy metal in the leaves and its effect on the medicinal plants studied.

Methods: Five different medicinal plants (*Vitex doniana*, *Cnestis ferruginea*, *Anthocleista djalonsensis*, *Ricinus communis* and *Manihot esculenta*) from polluted and unpolluted areas were studied to determine the effects of heavy metal pollution on their physical and anatomical features. Morphological features of the plants were examined using a planimetric method. Leaves foliar properties studied using photomicroscope and the metal load was determined by atomic absorption spectrophotometry.

Results: Leaves from the polluted site showed more affected features phenotypically than the control. Under elemental analysis, lead concentration at the polluted site ranges from 2.75 in *V. doniana* to 14.13 ppm in *R. communis*; nickel, 0.27 in *R. communis* to 0.5 ppm in *M. esculenta*, cadmium, 2.25 in *V. doniana* to 24.3 ppm in *A. djalonsensis*, zinc, 0.16 in *A. djalonsensis* to 1.58 ppm in *M. esculenta* and arsenic, 0.1 in *R. communis* to 0.7 ppm in *C. ferruginea* and 0.11 ppm in *M. esculenta*. In contrast at the control site, lead ranges from 0.00 in *V. doniana* and *C. ferruginea* to 5.52 ppm in *R. communis*, nickel, 0.13 in *C. ferruginea* to 0.21 ppm in *R. communis*, cadmium 0.00 in *V. doniana* to *C. ferruginea* 12.0 ppm, zinc 0.06 in *R. communis* to 0.16 ppm in *V. doniana* and arsenic 0.01 in *M. esculenta* to 0.52 ppm in *C. ferruginea*.

Conclusion: Only lead, cadmium and arsenic concentration in some plants were higher than the toxic concentration levels of their dry plants matters.

Keywords: Heavy metals, Leaf anatomy, Pollution, Medicinal plants, Quarry

Introduction

Medicinal plants occupy a vital position in the ecosystem because of their role as primary producers of the raw materials for many herbal formulations and popular supplements. They are also the major recipients of environmental pollution owing to their sessile nature, where as human and other animals can move away from sites with a compromised environmental status. Quarrying, crushing and transportation of limestone, zinc and substance within this community have been occurring for more than 7 yrs now at Ama-agu in Ishi-Agu Ebonyi state Nigeria. This results in the emission of fine white gray alkaline particulates dust around the farm lands and along the road that leads to the site. This dust consisting of slakes lines and flying dust, is predominantly deposited in the dry season. Evaluating the effect of pollution on medicinal plants is important to check the effect of air pollution on medicinal plants and other plants in general. Nigeria as a developing country, suffers from a pollution burden associated with the its increasing human population, vehicular traffic, infrastructure and industry, which has negative implications for the sustainability of human,

animal and plant communities [2]. Thus, it is necessary to access medicinal plants growing in polluted environments to determine their heavy metal load and protect them, given that only healthy plants can achieve the desired therapeutic response.

The experimental site used on this study is located at a hill top vegetation side of Ishia-Agu Ebonyi state Nigeria. Because of the major system and organs of the plants are exposed to the atmosphere, any surrounding air pollution can potentially impact on plants health. Some plants are sensitive to this; some are affected only minimal while others are tolerant. This research intended to provide a deeper understanding of toxic effects of quarry activities on medicinal plant, which could be useful for herb dealers and the public. It will create a standard for evaluating herbs before use and facilitate the appropriate choice of areas for herb cultivation.

Materials and methods

Plant extract preparation

Five different medicinal plant species (*Vitex doniana*, *Cnestis ferruginea*, *Anthocleista djalonsensis*, *Ricinus communis* and *Manihot esculenta*) were selected from the immediate vicinity

of the quarry site at the *Amagu* area of Ishiagu, Ebonyi State, Nigeria, as test plants. The test site is there farming and wild game environment that is about 10 to 15 min drive away from where villagers have settled. Another site with similar ecological conditions but not near any industry, quarry, or road was selected as a control site. This is located at *Amozara* village where people are settled and surrounded by vegetation. The medicinal plants were identified and the voucher specimen (*V. doniana* InterCEDD/16079, *C. ferruginea* is InterCEDD/701, *A. djalensis* is InterCEDD/1603, *R. communis* InterCEDD /160970 and *M. esculenta* InterCEDD /16042) at the International Center for Ethnomedicine and Drug Development, (InterCEDD) Nsukka, Enugu-State, Nigeria. Some of the plants were studied fresh. Whereas others were dried at room temperature for 2 d and ground for further experiments.

Macroscopic analysis

Leaf area/size and leaf length were determined using planimetric method. The leaf length and stalk- length were determined using a meter rule on a standard graph. Leaf area was determined by placing the entire leaf surface upside down on standard graph paper calibrated in 1 cm² and traced round with a pencil. Each cm³ that falls within the areas covered by the leaf surface that is not up to half of a cm³ were discarded in the count but all the cm³ within counted [3].

Microscopic analysis

A 1 mm³ section of the leaf was cleared in a solution of chloral hydrate, after which it was stained with 1% phloroglucinol, mounted on a micro slide using chloral hydrate as a mounting agent and heated until boiled. The preparation was allowed to cool and then mounted on a photomicroscope, using a 4mm objective (X400 magnification). The upper and lower epidermis was observed,

the stomata and other features were characterized and resultant image were captured using a high resolution digital camera [4]. This experiment was carried out on both test and control sample.

Elemental analysis

Acid digestion of the plants leaves was performed by weighing 2 g of a crushed and powdered portion in china dish, followed by heating in an oven at 110 °C to remove moisture. After charring the dried sample was added to a clean dried crucible and transferred to a muffle furnace, ashed at 650 °C for 3 h, and cooled in a desiccator. A total of 10 ml distilled water and 5 ml of 30% HCl were added followed by transfer of a 50 ml volumetric flask, and then dilution to 100 ml volume with distilled water, an aliquot of the solution was used for determination of the concentrations of lead, zinc, nickel, cadmium and arsenic in the solution [3,7, 8].

Results and discussion

The physical features of the test and control plant leaves are displayed in (Figure 1). Macroscopic / morphological examination of the leaves of experimental and control site gave the morphological and characteristic of medicinal plants. The results achieved by organoleptic evaluation and various botanical examination techniques, revealed differences in physical properties of medicinal plants grown in heavy metal and non heavy metal environment. This analysis recorded small surface area of the leave, yellow colouration and also reduced length of stalk in Test plants. The features were noted upon observing the specimen with the aid of a magnifying lens, as well as evaluation of the specimen using other sensory characters. The macroscopic analysis is the simplest and quickest indication for identity, purity or quality control when compared with official monograph.

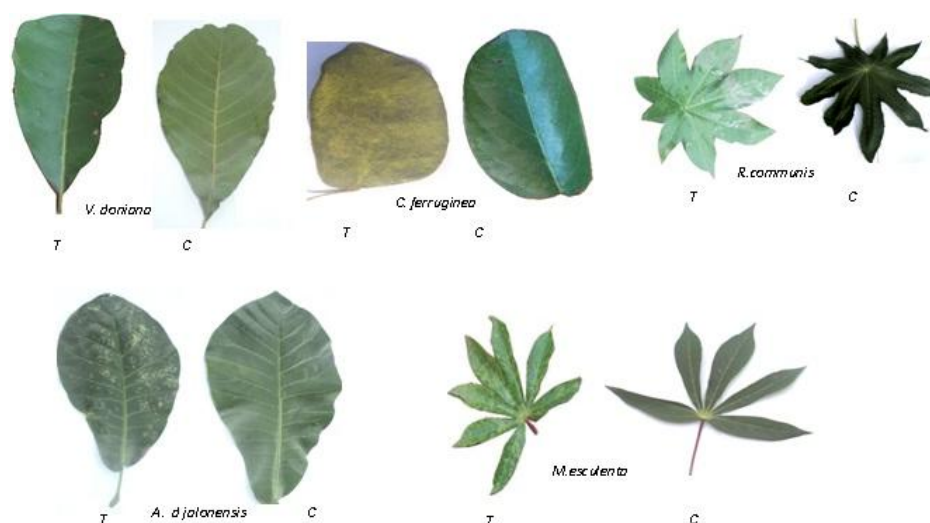
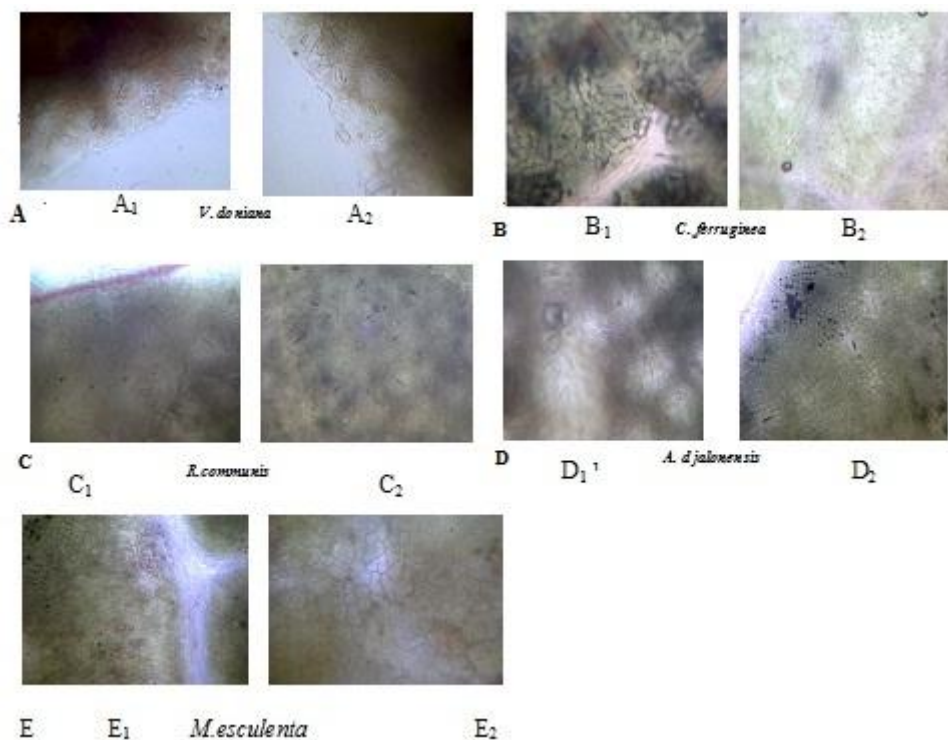


Figure 1: Macroscopic/morphological examination of plants leaves [13]. T = Test, C= Control



A,B,C,D,E = Numbers of slides, Slide A₁, A₂, B₁, B₂, C₁, C₂, D₁, D₂, E₁, E₂, = Test and Control plants respectively

Figure 2: Scanning electron microphotographs of leaf surfaces [9]

Microscopic analysis identified the stomata present in the medicinal plants. The features of fresh transverse section of the leaf identified gave the anatomical characteristic of the tissue obtained by transverse, radial and longitudinal sections represented in figure 2. It produced the number of stomata present in a particular leaves. This helps to know the photosynthetic ability of the medicinal plant in a particular environment. Slide (A₁) *V. doniana* is showing deformed epicellular wax and closed stomata, in which A₂ shows smooth wax with open stomatas. (D₁) *A. djalonsensis* showing striated epi wax and few stomata, while D₂ is showing healthy epicellular cells and high stomatal frequency. (B₁) *C. ferruginea* has healthy and robust wax, while B₂ shows damaged, bleached wax and closed stomatas. (C₁) *R. communis* gave less stomata frequency, swollen epicellular wax and closed stomatas, C₂ has healthy epiwax and many stomata opening. (E₁) *M. esculenta* shows bleached epicell and closed stomatas but E₂ has smoth wax and open stomatas.

Microscopic examination of the control plants showed multiple stomata, smooth wax, and large pores that were not swollen in contrast to those in experimental plants at both the upper and lower epidermis. This could have been associated with the plants trying to control the absorption of metal load (pollutants) and at the same time narrowing their stomatal pores in attempt to reduce aspiration of water, which limits photosynthesis. At the upper and lower epidermis, *M. esculenta* did not show recognized stomatal features present

at both experimental sites but the epithelia cell wall of the test plants was damaged. This suggests sensitivity to the environment as compared to the physical features of the leaves. When *R. communis* was compared with the control, it did not show a difference at the upper and lower epidermis which could have been due to its tolerance of the environment.

At both the upper and lower epidermis, *C. ferruginea* revealed few stomatal openings and closed pores when compared with the control. This was seen in the features of the leaves; *V. doniana* proved to be tolerant to the environment because, of their great stomatal frequency and more pores but rough epicellular wax when compared with the control. The efficiency of stomatal opening for carbon dioxide uptake and water transpiration determined not only by the sizes of opening but also by the number of stomata because more stomata can take up more carbon dioxide and transpire more [9].

The test plants leaf area, stomatal frequency, leaf length and leaf stalks measured gave less value than in control plants. This was possible due to the size of control plants leaf which was bigger than the test plants respectively as seen in figure1 [10].

This study examined the effects of quarrying activities and heavy metal pollution on the physical and anatomical properties of medicinal plants. The results showed significant changes in some physiological and biochemical properties of

the plants based on the physical and internal features that the leaves of these plants displayed. The high concentrations of the studied elements (zinc, nickel, cadmium, lead and arsenic) in some dry matter of the studied plants compared with those of the control plants were evidence in the variations in morphological and analytical features. This agrees with the findings of other researchers that pollutants can cause leaf injury, stomatal damage, premature senescence, decreased photosynthetic activity, disturbed membrane permeability, reduced growth and poor yield in sensitive plants species [13]. Some *V. doniana* individual had spots on their leaves which where as a result of fungi, bacteria, and insects; these always occur in with lime dust due to reactive oxygen that acts on the surface of the leaves with moisture. The leaves from the polluted site were tougher than those from the control site, *C. ferruginea* showed the presence of lime stone dust on the surface of its leaves

which even covered the mid-ribs of the leaves. The leaves also showed yellow coloration due to lack of photosynthesis which might have occurred due to the leaf area being covered with dust for a long time. At the polluted site, *A. djalonsensis* had smaller surface area, less green leaf coloration and reduced stalk length. In addition at the same site, *R. communis* had reduced area; bleached patches; and *M. esculenta* had yellowing of the leaf venation, tough texture of the leave and insect bites. The results of foliar examination provided details of the macroscopic features and anatomical properties of leaves from the polluted site. This revealed visible injuries and changes in the external structures of the leave as well as, features suggestive of stress, including a reduction in leaf area. This could have been due to poor air quality in the environment, stress from water loss, and changes in air chemistry which affect plant functionality.

Table 1: Morphological and microscopic comparative analysis of leaf length, leaf area, stalk length, and stomatal frequency

Sample	Family	Site	LL(cm)	LA(cm)	LS (cm)	SF
<i>V.doniana</i>	Vebernaceae	T	10.0 ± 0.1	85.0 ± 0.3	2.5 ± 0.2	3 ± 0.3
		C	14.0 ± 0.20	106.0 ± 0.2	4.0 ± 0.3	7 ± 0.1
<i>C.ferruginea</i>	Connaraceae	T	22.0 ± 1.0	30.0 ± 0.3	1.7 ± 0.3	3 ± 0.3
		C	32.0 ± 0.3	75.0 ± 0.2	3.0 ± 0.3	6.0 ± 0.3
<i>A. djalonsensis</i>	Loganiaceae	T	40.0 ± 1.0	870.0 ± 0.4	6.0 ± 0.3	4. ± 0.2
		C	64.8 ± 0.3	1350.0 ± 0.5	9.0 ± 0.2	14. ± 0.3
<i>R.communis</i>	Euphorbiaceae	T	16.4 ± 1.0	450.0 ± 0.3	13.0 ± 0.3	2.0 ± 0.3
		C	20.7 ± 0.4	605.0 ± 0.2	19.5 ± 0.2	6.0 ± 0.2
<i>M.esculenta</i>	Euphorbiaceae	T	12.0 ± 1.0	370.0 ± 2.0	12.5 ± 0.2	2.0 ± 0.3
		C	17.0 ± 1.0	420.0 ± 1.0	15.0 ± 0.3	4.0 ± 0.1

Values are mean ± SEM, n=3, SF= Stomatal Frequency, LA= Leaf Area, LL = Leaf Length, LS = Leaf stalk

Lead

Lead in plants is considered to have reached a toxic concentration at 5 mg/kg [8]. The graph presented in figure.3 shows the most prevalent heavy metal in the environment and medicinal plants with the highest concentrations of metallic components. Lead was most abundant followed by cadmium, arsenic, zinc and nickel but the medicinal plants that harbored the most metal were *A. djalonsensis* and *R. communis*.

Among the metals, Lead is absorbed rapidly and always move to the aerial parts of the plants causing visible changes to plants. *A. djalonsensis* (7.58 mg/kg), *C. ferruginea* (3.45 mg/kg) and *V. doniana* (2.75 mg/kg), in the polluted plants, whereas in unpolluted plants, lead was detected only in *R. communis* (5.52 mg/kg), *A. djalonsensis* (1.38 mg/kg) and *M. esculenta* (1.03 mg/kg). However the concentrations of lead in both test and control samples were within the accepted plant concentration level (ACL) of herbal metal contaminants. The presence of higher values for the test samples from the polluted plants suggested that pollution had caused this

because lead is associated with oxidative stress in plants as a result of its role in the elicitation of antioxidants from plants [5].

Nickel

Plants grown in Ni contaminated soil and media show various responses and toxicity symptoms including retardation of germination, inhibition of growth, reduction of yield, induction of leaf chlorosis and wilting. Disruption of photosynthesis, inhibition of carbon dioxide assimilation as well as reduction in stomatal conductance also occur [12]. Therefore, Ni may compete with these metals in absorption and transpiration processes. Via such competition, Ni at high concentrations may inhibit the absorption of these metals, decrease their concentration and even lead to their deficiency in plants. This may affect important physiological features in plants despite this metal being present at adequate concentrations, in the environment adversely affecting the internal and external structures of the plants [6] as shown in figure.2. The presence of nickel in all of the plants was disproportionately low at both polluted and unpolluted site. At the polluted site, *M. esculenta*

had the maximum concentration of nickel (0.54 mg/kg), followed by *A. djalonensis* (0.513 mg/kg), *V. doniana* (0.430 mg/kg), *C. ferruginea* (0.32 mg/kg) and *R. communis* (0.27 mg/kg). When this result was compared with the concentration in experimental plants, it was higher than in the control plants although both were within the accepted plant metal concentrated level which is 110 mg/kg [1]. It is well

known that other metals beside Ni interfere with essential metal ions such as K, Na, Ca, Mg, Fe, Cu, Zn, and Mn which are essential for plants. At the unpolluted site nickel level ranges from 0.23 mg/kg for *V. doniana* followed by 0.21 mg/kg for *R. communis*, 0.18 mg/kg for *A. djalonensis*, 0.18 mg/kg for *M. esculenta* and 0.13 mg/kg for *C. ferruginea* showing lower level than at polluted site.

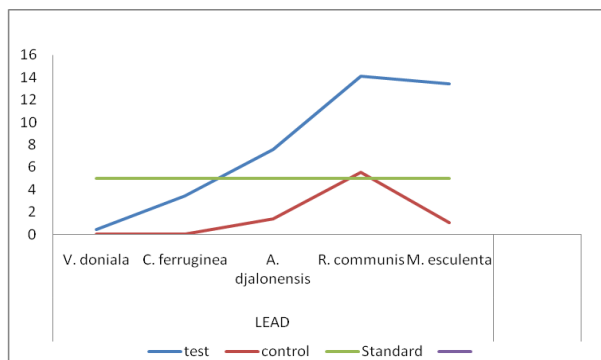


Figure: 3a

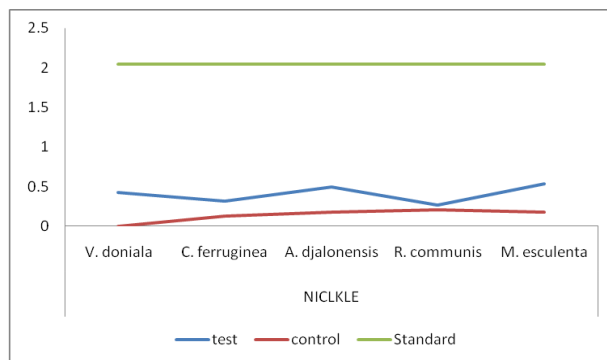


Figure: 3b

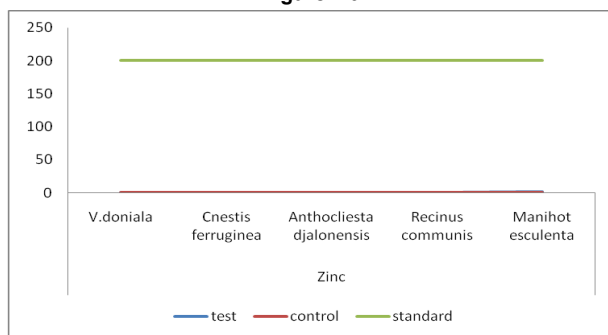


Figure: 3c

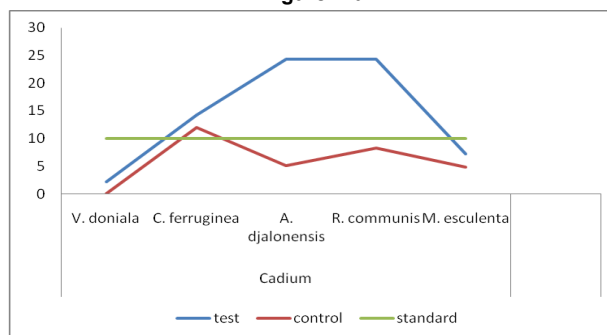


Figure: 3d

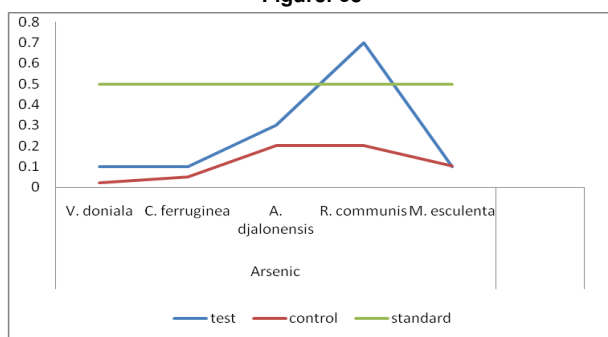


Figure: 3e

Fig 3 (a-e): Concentrations of heavy metals at test and control sites

The graph illustrated under figure 3 showed most prevalent heavy metal that occurred in the environment and medicinal plant with highest concentration of the metallic components. Lead occurred most followed by cadmium, arsenic, zinc and nickel but medicinal plants that harboured the metallic content mostly is *A. djalonensis* and *R. communis* [10, 14, 11].

Zinc

Zinc occurs in plants as a free ion, as a complex with a variety of low molecular weight compounds, or as a component of proteins and other macromolecules. In many enzymes, zinc acts as a functional, structural, or regulatory cofactor; a large number of zinc-deficiency disorders are associated with the disruption of normal enzyme activity (including that of key photosynthetic enzymes). Zinc deficiency occurs when plant growth is limited because the

plant cannot take up sufficient quantities of this essential micronutrient from its growth medium. It is one of the most widespread micronutrient deficiencies in crops and pastures worldwide and causes large losses in crop production and crop quality. Zinc deficiency also increases membrane leakiness because zinc-containing enzymes are involved in the detoxification of membrane-damaging oxygen radicals. The zinc in plants is considered to have reached a toxic concentration at 200 mg/kg [12]. Based on the results of this

study, the highest concentration of Zn at the polluted site was found in *M. esculenta* (1.58 mg/kg), followed by *C. ferruginea* (0.3 mg/kg), *A. djalonsensis* (0.10), *V. doniana* (0.08 mg/kg), and *R. communis* (0.06 mg/kg). At the unpolluted site, the highest concentration was found in *A. djalonsensis* (0.16 mg/kg), followed by *V. doniana* (0.15 mg/kg), *R. communis* (0.11 mg/kg), *M. esculenta* (0.07 mg/kg) and *C. ferruginea* (0.07 mg/kg). The medicinal plant with the highest level of zinc contamination was *M. esculenta*, which might have been the cause of yellowish venation of the leaves in some plants. This showed this plants sensitivity to the quarry environment despite the zinc concentration being within the ACL. This observation; may be the cause of the poor stomatal opening at both the upper and lower epidermis of the plants. Some of the visible deficiency symptoms include chlorosis, the yellowing of leaves, which is often observed at the veins in some species. Young plants are the most affected as the development is impaired, but in already developed plants, only the leaves become chlorotic. Due to zinc-deficient dicotyledons often have shortened internodes, leading to clustering of the leaves on the stem [13]. Stunting of plants, may occur as a result of reduced growth or reduced internode elongation. Dwarf leaves featuring small leaves that often show chlorosis, necrotic spots or bronzing; and malformed leaves are often narrower or have wavy margins. Yellowing leaf venation was identified in most *M. esculenta* located around the quarry site. The estimated safe and adequate daily intake of zinc is known to be within their respective ACL effect studies.

Cadmium

The antioxidant activity of plants is increased at a lower concentration of cadmium, but a higher concentration, leads to decreased activity of antioxidants. Cadmium in soil inhibits transmission or uptake of other metals such as iron and zinc [12]. Deficiency of essential metals causes poor growth of roots and shoots. Excessive cadmium in plants leads to reduced carbon fixation, lipid peroxidation reactions in DNA and reactions with protein which eventually leads to poor growth of medicinal plants.

Cadmium was also present in all the plant species from the polluted site and its concentration ranges from 7.26 mg/kg in *M. esculenta* to 14.3 mg/kg in *A. djalonsensis*. Its peak concentration in *A. djalonsensis* (14.3 mg/kg) was higher than the ACL of cadmium 10 mg/kg [7]. Its concentration was about 70% of the test plants, but the minority (30%) had cadmium content within acceptable limits. An increased concentration of cadmium may inhibit the metabolism of iron in plants causing chlorosis and reduction in photosynthesis. In terms of internal structures of these plants, they had low stomatal frequency, bleached rough and damaged epicellular wax. The cadmium levels at the control site ranged from 4.75

to 8.25 mg/kg, although cadmium was not detected in *V. doniana*.

Arsenic

The ACL of arsenic is 0.5 mg/kg [3], but in this study test plants showed arsenic contents ranging from 0.1 to 0.7 mg/kg where the highest content of 0.7 mg/kg was found in *R. communis*. The values observed at the control site ranged from 0.02 to 0.2 mg/kg which were lower than the values observed at the polluted site. At the polluted site, *C. ferruginea* was a good example of the features of leaves covered by quarry particulates matters. This matter covered the surface area of the leaf, causing poor leaf expiration to occlusion, which in turn reduce photosynthesis.

The result of this study, confirms that the internal and external structures of medicinal plants around quarrying site in Ama-gu Ebonyi state Nigeria are affected by exposure to an environment polluted by heavy metal from the industry.

Conclusion

A standard for evaluating herbs before use and how to choose areas for herb cultivation were also suggested by this research. Drug law Agencies in Nigeria should not only focus on regulating the law guiding orthodox medicine but medicinal plants inclusive to save the masses from taking contaminated herb. The health of medicinal plants can be improved if ethnomedical practitioners are aware of the need to cultivate herbs outside industrial areas. To minimize the effects of pollution which may degrade the medicinal plants and possibly affect their therapeutic efficacy awareness has to be created. The result of this study, confirms that the internal and external structures of medicinal plants around quarrying site in Ama-gu, Ebonyi state Nigeria are affected by exposure to an environment polluted by heavy metal from the quarry site.

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Conflict of Interest: None declared

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